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COVER:

Used in a nutrition education program in Ghana, this demonstration readily conveys a simple message: fresh eggs don't float. (UNICEF photo by Paul Almasy.)

IN THIS ISSUE....

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MASS COMMUNICATIONS IN NUTRITION IMPROVEMENT

INTRODUCTION

The wide prevalence of malnutrition, the severity of its effects in terms of human potential and the complexities of the necessary actions to prevent malnutrition underline the important role of communication activity in contributing to nutrition improvement. Nutrition improvement requires changes in behavior and such changes in turn require awareness of the benefits that may be obtained from acceptance of new ideas, abandonment of existing practices, or simple modifications or adjustments in food related habits. The complexity of malnutrition and the difficulties associated with attempts to change behavior make it essential that the ways and means of conveying correct and appropriate information to people are carefully thought out, developed and utilized. This is particularly important in changing behavior associated with food consumption.

In June 1973, the PAG considered the subject of mass communications in nutrition education at its 21st Session and issued PAG Statement No. 27 setting forth the objectives of nutrition communication activities and the basic principles and problems in organization, implementation, research, evaluation, and training in this area (PAG Bulletin Vol. IV, No. 1). The potential importance of mass communications in nutrition improvement efforts led the PAG at its 22nd Session in June 1974 to recommend that an ad hoc Working Group on this subject be convened. This decision was especially timely in view of the urgency attached by the World Food Conference (November 1974) to accelerating efforts to improve nutritional status in developing countries. The World Food Conference Resolution V, paragraph 4 states "that Governments include nutrition education in the curricula for educational programs at all levels and that all concerned in the fields of agriculture, health and general education be appropriately trained to enable them to further the nutrition education of the public within their respective domain".

The PAG ad hoc Working Group met at the United Nations Headquarters in New York on 24 - 28 November 1975 to discuss the shortcomings of the current efforts in the use of mass communications in nutrition improvement and develop a guideline for formulating operational strategies*. Edited version of some of the documents, and case histories presented by the participants are published in the following pages.

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*The report of the meeting will be issued separately.

THE ROLE OF MASS COMMUNICATION IN NUTRITIONAL IMPROVEMENT PROGRAM

Joseph Ascroft*

"Mass communication" brings to mind the print and electronic mass media of communication, which have long been held to have a powerful potential for accelerating development in less developed countries (LDCs). Yet, research shows that interpersonal channels remain far more important for producing behavioral change. But by accepting this generalization, we give up too easily on the potential of mass-media communication.

To be sure, a number of factors act to limit mass-media potential. For example, the mass media in LDCs achieve primarily only limited penetration deep into the rural areas where the bulk of the population is located. TV is restricted to urban areas, by virtue of high receiver cost and lack of rural electrification. The print media also are largely urban. In addition, radio is not yet in every rural home. We must investigate why the mass media have had such limited effects on the people they do reach and to question the notion that mass-media effects are limited to awareness-creation instead of direct adoption of innovations. The fault may lie with the practitioners, rather than the media, because commercial advertising seems to be immune from the awareness-generalization. Most media advertising spots are designed to elicit direct adoption, rather than mere awareness of commercial products.

Traditionally, the field of diffusion of innovations distinguishes between two broad categories of communication based on the media they use. Those using predominately face-to-face media are known as interpersonal communicators, who, in the rural context, generally are extension agents. Those who use print and electronic mass media usually are called mass communicators. However, in LDCs, these two role-players are totally different from each other, in terms of background and training. Extension agents are generally rural-oriented, with a strong background in such disciplines as agriculture, health, education, or community development. They may have received a modicum of practical training in audio-visual methods of field extension of innovations, but little training in ways of changing basic behaviors of people. They are, first and foremost, technologists, innovations experts.

Mass communicators, on the other hand, are generally urban-oriented, trained in the techniques and technology of media management and operation, or in the production of messages for mass consumption. They may have some theoretical training in mass-media techniques and tactics for changing people's beliefs, attitudes, and behaviors, but little practical training. They, too, are technologists, mass media experts.

Both these roles are necessary, but there is a missing role, which emphasizes the knowledge and skills of how to change people's behaviors in desirable directions. Such a role already exists in commerce and industry, where specialists in communications, called commercial advertisers, are skilled in knowing how to motivate individuals and masses to adopt the manufacturer's new products.

An appropriate label for this role-player in our diffusion process is communication expert. The innovation expert and the mass media expert play different, yet complementary roles. The communication expert brings the two into a fruitful, dynamic relationship.

The Innovations Expert

Innovations experts are the first crucial link

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in the diffusion process. Agriculturalists are considered experts in the innovation "hybrid maize", because they understand its potential for multiplying yield per acre, and also are skilled in cultivation techniques from field preparation and early planting to storing and marketing. Similarly, nutritionists are innovations experts who understand the health-promoting advantages of protein-rich foods from oilseeds and cereals and also are skilled in preparing such foods for weaning or supplementary feeding purposes.

In LDCs, it has generally been left to innovations experts to carry out the interpersonal communication of innovations in the field. To do this, they "train" in methods of extension, ranging from preparation of simple audio-visual aids to techniques of field demonstration, plus group and interpersonal work. The extension education curricula, however, are more appropriate for developed countries, where many of the problems unique to LDCs are never encountered. They are not burdened with populations which are overwhelmingly rural, uneducated, illiterate, and traditional in their outlook. They do not suffer the problems of multiple tribes, languages, and extended family systems. They are not victims of poor roads, mail services, and telecommunications. They are not faced with small-acreage, subsistence farms and underdeveloped marketing systems. They seldom have to worry about inadequate distribution of the goods and services they recommend. And, above all, field agents rarely labor under 2,000-plus caseloads.

Hence, comprehensive curricula and techniques to take account of such constraints have not been developed, and LDCs have not been able to offer a utilitarian framework for training extension personnel.

The Mass Media Expert

The role of a mass-media expert encompasses many skills, ranging from the operation and maintenance of cameras, projectors, recorders, and printing equipment to the production of a wide variety of sensory stimuli, including words, pictures, and sounds. Their purpose is primarily to satisfy the voracious public appetite for consumer information and entertainment. This orientation seriously limits the purview of mass-media experts in present-day LDCs, where the accent is on news, information and entertainment, with little accent on producing desired behavioral change.

Of course, there are exceptions. The most important are the radio farm forums, where small groups of rural folk are gathered under the leadership of an innovations expert to listen to regularly scheduled radio programs that promote the adoption of relevant innovations and provide basic education, such as literacy training. The programs serve as an impetus for group discussions, which often result in feedback reports and questions to the centrally located broadcasters, comprised of mass media and innovations experts. The media forums are an attempt to combine the awareness-creation propensities of the mass media with the behavioral-change benefits to face-to-face communication in small groups. But their impact is limited for logistical reasons, including poor signal reception in some areas; the expense of distributing and maintaining a large number of receiving sets; the absence of "instant replay" facilities, which forces listeners to remember detailed messages on the basis of hearing it only once; and the problem of language selection in multilingual societies.

Moreover, the number of listening groups possible is constrained by the number of innovations experts available for leading discussions. This is aggravated by the fixed time of radio broadcasts, which presupposes that an entire population will find it convenient to be at a given place at a given time. Only a small proportion of the rural population is thus ever able to get into the forums.

Finally, except for radio forums, coordination and understanding between innovations experts and mass media experts often leave much to be desired, because one is based in a city, the other in a rural area.

As a result, innovations experts often complain that mass-media experts do not understand their needs; mass-media experts frequently cirticize innovations experts for their use of abstruse, esoteric language of science to describe their innovations. A reporter's attempts to simplify language often bring complaints by innovations experts that mass-media experts do not know what they are doing. It is small wonder that the primary effects of the mass media upon rural audiences often are limited to awareness-creation. Clearly, there is a need for a third role-player, whose job is to bridge the communication gap and absorb the functions that seem to fall between the bipolar groups.

The Communication Expert

The immediate problem is not to beef up the curriculum of those currently undergoing training via massive infusions of communication theory and practice, but to retrain those already practicing in the field. The communication expert, ideally, is neither an innovations expert nor a mass-media expert, but is one with a general understanding of these two areas of expertise. He works at once with an agriculturalist or a nutritionist or perhaps a family planner, and with a radio specialist, a film producer, or a print-media expert. He takes these skills as given, and seeks to add his own specialized skills to bring them into dynamic, functional relations with each other. The main conceptual tools that define the communication expert's sphere are comprehensive knowledge and skills in such areas as:

1. Characteristics of innovations as perceived by potential adopters;
2. Construction of change-producing interpersonal and mass-media mesages tailored to the needs of specific audiences;
3. Selection of credible and acceptable message sources;
4. Selection of specific media and channels to create specifically intended effects; and
5. People-involvement in helping to plan and implement their own development.

The Characteristics of Innovations

An innovation is an idea, a practice, or an object that is perceived as new in the eyes of potential adopters who are being introduced to it for the first time. Communication experts focus upon how that innovation will be perceived by potential adopters. They must know about those general characteristics of innovations that provide a basis for evaluating the diffusability of a given innovation. These are:

1. Relative advantage - the degree to which an innovation is perceived by potential adopters as being more useful and productive than the idea it supercedes;
2. Compatibility - the degree to which the innovation is perceived as consistent with existing values, past experiences, and needs;
3. Complexity - the degree to which the innovation is perceived as being relatively difficult to understand and use;
4. Observability - the degree to which the results of the innovation are obvious to others; and
5. Trialability - the degree to which an innovation may be tried out on a limited, risk-free basis before the potential adopter needs to commit himself to full adoption.

The relative advantage of a nutrition innovation may be perfectly clear to trained experts, yet obscure to their clients, because, they are too sophisticated. Appropriate symbols and message treatments must be found and pretested to make sure they fit the perceptions of intended adopters.

Some nutrition innovations are hard to handle in terms of compatibility when they impose a host of new tastes, smells, textures, and appearances. Ways must be found of making new foods appear less strange in the eyes of intended adopters.

The benefits from eating protein-enriched foods have little immediate observability to others. Before-and-after pictures need to be produced to demonstrate improvement over time resulting from protein-enriched diets.

Preparing new foods, such as baby formulae and balanced diets, may be relatively complex to understand and use because of involved instructions and hard-to-translate terms.

Some way must be found to reduce complexity to easy, one-step-at-a-time dimensions.

Trialability of a new food is often hampered by its nonavailability on the market, or to the difficulty of obtaining handy sample sizes for a small-scale trial. Some way should be found to facilitate risk-free trials.

A useful way to determine how potential adopters view these five characteristics is by participant-observation with representative test cases. From observation and discussion, the observer can collect data that may enable him to plan appropriate action to take account of the objections and difficulties.

Message Construction

A message is a symbolic representation of a concept that is not amenable to public observation. An in-the-mind concept must be externalized to become observable. This is the area of message construction, which has at least two main components; symbol selection and message treatment.

For instance, two nutritionists teaching different groups of rural women about balanced diets may have totally different approaches. The first may use technical terminology, such as protein, calorie, and carbohydrates. This scientific language may go over the heads of her illiterate students. The other could depend on long-winded descriptions and rambling analogies. Same concept, different symbol selection, though each represents an intuitive, rather than a carefully premeditated, approach. Neither method may be successful.

Message treatment refers to the persuasive component of a message that serves as motivational drive for behavioral change. There are many ways to produce such change, each with its own peculiar advantages and disadvantages. A communication expert is trained to assess and avoid a situation where losses are likely to be more assertive than gains.

Source Selection

Who communicates to rural folk is often more important than what is communicated. Given the same symbol selection and message treatment, one source may be believed and another rejected. Some sources are deemed more credible than others because of their perceived trustworthiness, competence, or social acceptance. In LDCs, the social acceptability of a source is frequently more important in credibility than are competence and trustworthiness combined.

For example, change agents in LDCs (nutritionists included) tend to be very young, in comparison to their clients. However, tradition in many LDCs--especially in child-rearing and food-preparation--dictates that the old teach the young. Suddenly, the old find themselves sitting at the feet of the young, who are unprepared for the task and adopt a haughty, authoritarian style of presentation. This causes the old folks to reject both the young people and the innovations they espouse, no matter how beneficial these may be.

The trained communicator is sensitive to the need to find acceptable sources for the messages he wishes to transmit. A useful strategy is to recruit rural persons, already perceived as credible, to serve as primary sources of communication. Local leadership figures may be selected, and given direction in the fundamentals of the innovations to be diffused to serve as discussion leaders. When technical detail needs to be added, or if questions arise that the leader cannot handle, the young experts, who stand by as resource people, are summoned to fill in the gaps and conduct demonstrations.

Channels and Media Selection

Channels and media are the means by which sources are linked with their audiences. The association may be interpersonal or mass-media communication.

Interpersonal communication enables the source to control the communication situation by monitoring feedback, which allows him to

modify his approach as he goes along. However, the size of audience that can be reached is very limited.

Mass-media communication, with the possible exception of media forums, can command very large audiences. Under what conditions should one use interpersonal communication, mass-media communication, or both?

The answer usually depends on the complexity of the innovation to be diffused. Simple innovations lend themselves very well to mass-media diffusion. As the innovation becomes more complicated, interpersonal channels are more valuable.

Commercial advertisers use the mass media as primary sources of behavioral-change communication, not merely to achieve awareness, but to gain direct adoptions of simple products, such as soft drinks, cigarettes, cornflakes, and detergents.

More complex innovations require more involved diffusion techniques, comprising a combination of mass media and interpersonal communications. Understanding that a new seed variety has the potential to triple the yield is a relatively simple matter. Learning how to use the new seed, with all its ramifications of cultivation, is considerably more complex. Similarly, understanding the notion that diets should be balanced is more easily achieved than learning how to prepare that diet.

In commercial advertising, creating understanding of the relative advantage of a new product is often handled through the mass media, whereas training people how to use it is left to salespersons and point-of-sale demonstrations. This makes for an effective combination of mass and interpersonal sources of communication. Similar procedures can be used to diffuse complex rural development innovations. Diffusion scholars postulate that an individual passes through four cognitive and behavioral stages before deciding to adopt or reject an innovation.

These stages are:
Awareness - when one learns of the existence of an innovation for the first time;
Interest - when one's fancy is stimulated enough to gather detailed information about the innovation;
Evaluation - when one assesses the viability of adopting the innovation in terms of one's values, capabilities, and needs; and
Trial - when one actually submits an innovation to a small-scale test to insure that it is worthwhile.

An innovation may be aborted at any of these stages. It is the business of the communication expert to arrange a strategy that leads the potential adopter systematically through each of these stages, one by one. The awareness stage is when people learn of the existence of high-yielding seed varieties, of ways to space children, and of certain foods to promote good health. Relative-advantage messages are often simple, and can be carried via radio, perhaps as advertising spots repeated regularly in the manner of commercials, and continuing throughout the remaining stages.

At the interest stage, more details are made available. But radio's utility is limited for this purpose; words alone are seldom adequate to describe an innovation, because they appeal only to the sense of hearing. TV is a good device for including the visual aspects, but it is usually restricted to urban locations. In rural areas, films, filmstrips, and slides shown by mobile audiovisual units are useful substitutes. Presentations should emphasize the relative advantage of the innovation in greater detail, and its compatibility with the values, past experiences, and needs of the audience. One way to do this is to make sure that the people, settings, and displays in the pictures are consistent with what is typically found in the surroundings of the audience.

The evaluation stage provides "how-to" details. An innovation is broken into a series of small, simple steps to reduce its complicated nature. Where some literacy exists, printed materials with pictures help potential adopters to evaluate an innovation.

It is more likely, however, that literacy is a problem, and the potential adopter will have to be helped to make evaluations through live demonstrations, field days, and question-and-answer sessions with informed sources.

The trial stage is one in which an innovation is subjected to small-scale testing, because it allows people to make mistakes on a small, risk-free scale rather than on a large, costly one. Certain key community people should try out the innovation under supervision in their home environment, using their own available equipment. Friends and neighbors may be called in to observe this trial and, perhaps, use the person they observe as a referent when later they try the innovation themselves, thereby multiplying the diffusion effects.

Thus, as one progresses from awareness to trial, one proceeds from a stage at which mass media predominate as the principle channels of communication, to a stage at which interpersonal channels are more important. The planning, guiding, coordinating hand throughout the entire process belongs to the communication expert.

People Know-how and People Involvement

The art of people know-how in the diffusion of innovations is identifying, utilizing, and involving that small segment of a population whose cooperation it is most crucial to acquire, to gain widespread adoptions of innovations. Not all individuals in a community adopt a given innovation simultaneously, and some of those who adopt early have significant leadership characteristics that make those who adopt later, usually the majority, follow their example.

Diffusion researchers generally agree that people can be classified into five categories: (1) innovators; (2) early adopters; (3) early majority; (4) late majority; and (5) laggards.

Innovators are the first 2 or 3 per cent to adopt an innovation. They are venturesome and eager to try out new ideas. This tendency, however, often makes them appear to be deviants, since innovations, by definition, are not normative to the community to which they are being introduced.

Early adopters comprise about the next 12 per cent of adopters, and are noted for the respect they command in the community. They are regarded as normal, stable leaders.

The early majority are the next 34 per cent or so of adopters. They are usually bereft of leadership, and seem unable to make up their minds until they have the example of the early adopters to follow.

The late majority constitutes roughly the next 34 per cent and includes the conservatives and skeptics who adopt innovations only when social system norms favor behavioral change.

Laggards are the last 15 per cent of adopters. They include the hard-core resisters to behavioral change, plus traditionalists who hold out to the bitter end before giving in to the pressures of a world changing around them.

Obviously the most important group to take into careful account early in the planning of a diffusion strategy is the leadership group, which includes both innovators and early adopters.

Innovators are clearly negative leaders because of their perceived deviancy. They are eccentrics, so the ideas they embrace, no matter what their relative advantage, are thus disregarded.

Early adopters, on the other hand, are positive leaders because they swim with the current, normative behavior. When they embrace a new, previously non-normative behavior, it places the community's seal of approval on it. Thus, who is better than the early adopters to involve in one's development initiatives?

How does one identify early adopters for a nutrition innovation that has not yet diffused to them? The answer lies in identifying early adopters for allied innovations that already have been diffused. In various communities

in Africa, some innovations, particularly agricultural, have spread to some extent. Research in Kenya shows that early adopters of one rural innovation tend to be early adopters in others, as well.

Even in communities where no innovations have diffused, it is possible to identify opinion leaders, who are generally included among early adopters, by eliciting from a representative sample of a community the names of those community members whom others wait for to adopt new ideas.

Among benefits to be gained from zeroing in on such a small, yet powerful, segment of a community is the potential for involving them in media forums, such as those described earlier. Media forums are primarily a means of allowing interest to build and evaluation to occur under supervision to insure favorable outcomes. Because of their leadership characteristics, early adopters should be used in such forums. If they become interested in an innovation, evaluate it favorably, try it, and eventually adopt it, chances are increased that others will follow their lead.

However, narrowing the target population to the early adopters renders radio an inappropriate medium for use in media forums, because it cannot be beamed only to early adopters. The same effect can be achieved by switching from radio to tape cassettes, which offer the flexibility of scheduling forums at times convenient to all. The same extension worker can meet with more groups per day than is possible with fixed-time radio broadcasts. Symbol selection and message treatment can be tailored for compatibility with specific language and tribal groups. Message sources can be changed, so that each listening group is exposed to a source it deems most acceptable. Cassettes can be played and replayed as often as is necessary, and they can be presented along with slides, filmstrips, and other sensory aids, so that the forums become truly multimedia.

The art of people know-how and people involvement in planning and implementing a diffusion program may be summarized in the following way: people know-how is the art of knowing the essential characteristics of people in different adopter categories and of how to use the special category which promises to have powerful diffusing effects on the majority of the community; and people involvement is the art of tapping into the native wisdom and organizational ability of that special category of people to help plan and implement a diffusion strategy compatible with the rest of the community.

Until communication experts have been trained and incorporated in program planning and implementation teams, and until strategies such as those described in this paper have been thoroughly tried out in field experiments, the case for mass communication as a viable means of accelerating the adoption of more productive, or health-promoting, innovations, must remain open.

VESTED INTERESTS AND FUTURE PERSPECTIVES IN MASS COMMUNICATION AND MEDIA

A Few Small Notes

Andreas Fuglesang*

The role of mass media cannot be understood and properly described if they are isolated from their national and international economic and cultural context. The capacity of a developing country to establish a mass media system and use it effectively for educational purposes--is strongly affected by inherent constraints in the international context. Education for nutrition improvement in developing countries tends to be dominated by a one-way flow of information from technologically advanced metropolitan centers through newspapers, radio, television, and films (1).

Rarely is more than a trickle of indigenous information about developing societies diffused through the mass media in developed countries (1). Studies are being done on the flow of information from the international news agencies (2) and on the flow of television programs (3) that confirm the one-way dominance. The broader framework of this phenomenon is the increasing control of information and information retrieval systems by major international firms and governments. This control comprises both the technology necessary for establishing national mass media networks and the message, the content, or programing which these networks carry. Decision makers in charge of social communication policies and mass communication policies in nutrition improvement in developing countries cannot make good decisions without an intimate awareness of this situation.

Up to 90 percent of the TV airtime in Africa is taken up by programs from the international information monopolies (4). Out-of-date films and TV serials no longer useful on domestic markets are dumped in developing countries for prices as low as $75 for a one-hour program. There are no budgets for local program production, which is bound to cost ten times as much. Once the technology is established, it is followed by the notion it must be utilized for continuous broadcasting (5). The system functions like a vacuum cleaner which sucks up whatever software is strategically placed in its proximity. Entertainment programs predominate. Documentary and news programs are almost totally absent. Most programs are alien to the local culture, and carry considerable information about food and nutrition that induces attitude change and has a habit-forming effect on its audiences.

In addition, explicit educational attempts are being made, in the form of advertising spots, for products ranging from vitamin pills and chocolate to infant formulas and reinforced milk powder. Developing countries, with high birth rates, are particularly vulnerable to such advertising. Child-feeding habits are particularly under attack. Milk powder is replacing breast-feeding. Scarce monies are exchanged for costly canned foods, instead of a balanced diet of traditional food-stuffs, etc. Nutrition educators with experience in developing countries seem to agree that the effects are disastrous, particularly in the highly exposed, spontaneous settlements in the urban fringes.

Television has expanded into developing countries primarily as a commercial medium. It has been paralleled by the

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international expansion of advertising agencies, which receive commission for the spots they buy on the air for their clients. This relationship has given a considerable boost to the establishment of television in Latin America, for example (1, 6).

Multinational firms today are marketing mass-media systems as total packages, including the technology (with monopoly on spare parts), personnel, programs, organization for attraction of advertising to the station, and provisions for training of local staff (7). The second phase is the purchase of part interests in local networks by foreign capital. This is not necessarily bad, but mass media cannot be treated as an abstract professional concept. If we recommend utilization of mass media for nutrition improvement, we are also recommending the mass media establishment per se.

It may be maintained that in countries like Zambia, Ethiopia, and others, independent educational broadcasting and television systems exist which are more suitable vehicles for nutrition education than are the general networks. This is true, but it should be noted that so far these institutions have been given a low priority. Private investors and multinational corporations, in particular, have dominated the field, often unhindered by alternative expertise recruited through aid agencies. This has had a considerable effect recently in some countries (Jordan and Ethiopia, notably); where specialists in educational broadcasting, now more generally recognized as an important aid priority, found it difficult to adapt to the method of operation established by the commercial firms (1). The ultimate and frightening (?) perspective for the mass-media developments is the new video technology, which, in the next decade, will take us all in its grip. Presently, a video cassette of 30 minutes costs about $125. The next step is the video disk, which may end up costing only a couple of dollars. It is a light, flexible, plastic disk which can be distributed as an insert in newspapers, for example. In other words, governments which are not prepared can lose entire control of their educational policy. Their countries can be flooded by educational programs of dubious value. The working group must take these circumstances into consideration. Strategies for mass communication in nutrition improvement cannot be developed in isolation from the reality in which they are supposed to function.

Models and Basic Research

It is difficult to accept the now almost traditional model which maintains that all psychology moves from change in knowledge level to change in attitude to ultimate change in behavior. This is parallel to the model for diffusion of innovations: from awareness to ultimate adoption. The theory on cognitive dissonance (8) seems to indicate that the opposite may well happen. People act and change behavior first, and then develop attitudes which justify and rationalize the behavior for themselves afterwards.

More serious objections can be raised regarding the idea of the early adopters and those generally called opinion leaders to which the development of mass communication theory is so closely linked. It is assumed that the majority of people do not receive their information directly from mass media, but from personal contact with the opinion leaders in their group. These latter individuals, in turn, expose themselves relatively more than others to the mass media (9). However, little attention seems to be paid to the fact that the Columbia researchers have themselves thrown doubt on their famous theory (10) and restructured it under the concept of shared interests. The flow of influence is not so much directed from highly interested people to people who are not interested at all, but from interested people to people of equal, or perhaps very slightly lower, interest. Shared interests, in short, appear to be a channel through which communications flow. The hypothesis of shared interests has also been tested in Sweden (11, 12).

Between 1964 and 1972, some 50,000 adults answered questions about their interests in 160 different topics and activities. Some of the

main findings are:
1. With increasing topical interest, there is increasing exposure for topical information--but not for information in general.
2. The increase of exposure for topical information is relatively independent of the type of channel. Exposure for personal, as well as for mass communication, increases with increasing interest.
3. Both advising and the information-seeking activities increase with increasing interest.

People's interests are--as the Columbia researchers have suggested--the channels through which communications flow. However, some explanations are necessary.
1. Interests are topical, and interests control all communications. Consequently, mass communication also is topical. It works selectively on topically interested sub-groups of the population.
2. Within the target of topically interested people, the information does not go in two steps, but in many steps, not only in one-way traffic, but both directions between interested individuals.
3. Different topics attract different numbers of people. Some topical interests are general (food and clothing); others are more specialized (buying/selling of shares, or 16 mm. filming). Therefore, the flow of communication is very broad in some cases and very thin in others.

The interpretation of shared interests is that the competence of the communicating man is restricted to the topics in which he is interested. Thus, every topic has a pre-determined target of recipients on the market (12). Mass communication has a long way to go before it has developed a comprehensive model. This indicates that great caution should be exercised in linking recommendations on strategy and guide lines for nutrition education through mass media to a model such as the innovation theory.

Admittedly, it may be the only choice, but the notion of shared interests allows for a much finer definition of target audiences to suggest new research in development work is becoming-and not unjustifiedly-less fashionable these days. However, research in this field is highly operational and tied to implementation programs. If this working group should suggest new undertakings, it may be desirable to concentrate on the field of shared interests. Who is interested in what within the field of food, nutrition, maternal and child health care? What are the significant topical interests within this field? How does the shared interest theory, developed as it is in sophisticated consumer societies, apply in societies of a traditional/transitional character, like LDCs? When treating the whole question of mass-media research and mass-communication programs, it may not be possible to separate the role of mass communication in nutrition improvement from the broader aspect of nutrition education, and that aspect again from the total aspect of general development education. Improvement of nutritional status is closely related to quality of life and fulfillment of the whole personality. But nutrition education has slim chances of succeeding unless it runs parallel to a growing consciousness--and conscience--about fellow men and the world (14, 15).

Experience in Sweden clearly confirms that mass media are most efficient when made part of multi-media campaigns (4). The "study circle" is the basis in these campaigns, and the mass communication input is radio or TV programs produced for the purpose. VTR and sound tapes are also used. Social consciousness is the general purpose of these study circles, and nutrition is among many topics treated. I believe that a useful strategy for mass communication in nutrition improvement may be found in programs of this sort.

Mass Communication and Illiteracy

Recent research indicates that mass media have a way to go in finding a graphic/pictorial style of message treatment suitable for illiterate audiences (16-20). Two Nigerian sociologists, Francis Okediji and William Ogionwo, say, after having tested a family-planning film and found that as much as 40 percent of a village audience

remembered its story only vaguely or not at all: "It is impossible to escape the feeling that it is necessary to devise special kinds of films for semi-literate rural audiences. An important factor is the ability of unlettered audiences seeing films for the first time to comprehend films which are forceful, clear and pertinent to their experience; it follows that films made for rural audiences should make a much greater effort to use the camera effectively to tell a story in pictures at a low pace and in a simple manner."

Social Marketing

Advertising is only one of a number of parameters in a marketing campaign for a product; such a campaign is characterized by a high degree of coordination of its various inputs.

Distribution, sales organization, sales promotion, public relations, advertising, price, product development, and packaging are equally important variables which are thoroughly researched, developed, and put into effect with a common objective. Often subobjectives are quantified for the individual variables. Operationally, marketing campaigns are very similar to implementation or intervention programs in development work. Interesting attempts have been made in designing models (21) for what is called social marketing, i.e., marketing of services and ideas, such as concepts for improved health and nutrition. Coordination, organization, and professional management are essential. Measured with this stick, most of the intervention programs may be described as amateurish.

The second resource which may be tapped in this industry is creative capacity, which is personalized in the graphic artist, the visualizer, and the copy writer. If totally sensitive to the culture, this level of professionalism can contribute to creating the images and ideas on which all mass communication depends.

Components in a Strategy

Availability of nutrition information is a necessity in any society. In industrialized countries, the model has usually been that motivated professionals and politicians have lobbied for the creation of an official food nutrition advisory council or information office, which is given the task of spreading nutrition knowledge in school curricula, in the mass media, in training institutions, in actual field work of such extension services as public health, community development, agriculture, in industries and large-scale farming connected with worker-feeding schemes, in women's clubs and societies, etc. Such information offices often can produce literature and educational material of high quality. In this total picture, mass media contribute to, but not replace, all other communication methods.

In the United States, in Scandinavian countries, and many other countries in Europe there are information offices or societies for such "causes" as cancer, cardiac diseases, dental care, food and nutrition, and a host of others.

Do developing countries need another pilot program? Does it serve a purpose to design and recommend elaborate strategies that may never be replicated on a national scale because the economic inputs are unfeasible and because nutrition is not considered to be important politically?

Aside from these, the following factors should be considered. The objective of a mass communication program in terms of knowledge level, attitude, or behavioral change is difficult to define quantitatively unless the total intervention program has a stated objective, such as change in nutritional status or food intake. The strategy should combine several research models in order to assess their relative practical applicability. Mass media should be recommended primarily as a single component in interpersonal communication, such as forums or study circles.

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SOME NOTES ON PLANNING COMMUNICATION SUPPORT FOR AN APPLIED NUTRITION PROGRAM

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Nutrition development is among the most complex, multifaceted, and behaviorally sensitive challenges in a national development program. For instance, neither improved production of nutritious foods alone, nor that plus improved, balanced consumption of nutritious foods, will complete the positive development cycle if poor environmental sanitation and health care maintain gastroenteritic and other disorders. Again, securing production of nutritious foods is not just a technical matter of which foods will grow in a given project area, nor only of communicating the needed cultivation practices to farmers and their families. There must be a balance of economic argument that will make sense to them. If the families are to start consuming more such new or increased-availability foods, the foods must either be compatible with their existing tastes and eating habits, or profound changes in their food behavior patterns must be brought about, and so on.

The targets set for an Applied Nutrition Program (ANP) may be stated clinically and quantitatively in terms of measurable improvement in the diet of a given community or of priority age groups in that community, against base-line data of specific nutritional deficiencies. Implicit in these stated target objectives are behavioral-change objectives, in which planned and sensitive human communication will have to play a significant role. The diet improvement will begin to be effected:

- When identifiable groups of people are helped to know and to understand certain things about their food production and consumption habits in terms that will make sense to them;
- When they receive motivation and information for new food production and consumption habits and techniques in forms they can understand, consider, become ready to adopt, and then sustain;
- When they receive medical and other health inputs, cultivation supplies and tools, and possibly initial financial assistance, through personnel who are adequately trained;
- To deliver these inputs sensitively, and in a coordinated manner, because these personnel understand and accept the need for coordination, and are supported by their superior authorities.

The work of the nutrition communication planner and implementer can begin only when these behavioral-change objectives have been very carefully defined for the total ANP. Precisely because the changes require other inputs from the ANP (medical, sanitary, agricultural, etc.), not just communication, feasible behavioral changes cannot be achieved by communication alone. On the other hand, it will not be possible to determine which behavioral changes will be feasible without careful consideration of the cultural, psychological, and informational factors already in the community with respect to food and diet.

The nutrition communication analyst and planner therefore need to be involved with all other specialists in the actual formulation of the detailed Applied Nutrition Program before preparing communication-support.

A mistake that has been made in many countries is to assume that the only people who will need to achieve behavioral changes for a successful ANP are the community itself--

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usually the rural villagers. But before the ANP even reaches the village, large numbers of other people will need to receive carefully planned bodies of information and even to adopt new ideas, because they will be key transmitters to the villages. In nutrition projects in various countries around the world which assumed that behavioral changes were needed only among villagers, it was found that, for example:

From the central ministry offices through their provincial and district branches, only a handful of all the civil servants who actually needed to be involved, knew anything about malnutrition, its causes and consequences, or the objectives and functional steps of the ANP itself. In some instances, this lack of information has even caused blockage of vital logistical support.

In a health ministry, key personnel serving the villagers did not really understand that an ANP required preventive, not merely curative, health measures.

Because of inadequate briefing and training, agricultural extension staff involved in, e.g., encouraging village poultry production, did not understand that a vital part of their role was also to try to persuade new poultry raisers to consume some of the eggs and meat in their own family, not sell all new produce.

The above are but some examples of the critical need to set behavioral objectives for all groups of people who can be identified as essential to the implementation of an Applied Nutrition Program, and to plan detailed communication-support efforts for those audiences as well as villagers.

Another weakness in nutrition programs, often identified only after a costly period of operation, concerns the training of those personnel who will carry the actual operations, down the infrastructure to the villages. Three broad observations may be made, in communication terms:

The project cadres must be trained as to the behavioral objectives and program, and not just in the technical facts about balanced diets. They must be made sensitive to the psychological factors and needs of the people whom the ANP is to help. They must be trained in interpersonal and group communication. They should learn fully what the traditional habits and attitudes regarding food and diet are for their project area and what lies behind these.

They should be trained in the appropriate, sensitive language to use in their work with villagers. They should know the special subtleties of traditional village terminology related to food, health, babies, pregnancy, etc.

They will also need advance knowledge of the economics of their project area, and advance understanding of how the nutritious food production and consumption behavior-changes to be sought will be affected by, and affect, that economy.

For necessary training in the technical facts of a balanced diet, their instructors must be equipped with sensible diet models. Careful consideration should be given to whether it is really necessary to provide training in diet in terms of precise and decimalized weights of given proteins, vitamins, etc. In many nutrition training courses that are not for medical personnel, use of these descriptives and minutely quantified models simply does not work, except for mechanical note-taking.

It has been proved that time and money spent on careful preparation of training materials--course notes, handouts, and especially audio-visual aids--pays major dividends.

Personnel who will be carrying out communication roles in the villages must be trained in and with the actual communication aids that will be issued to them. At least the first kits of village-level communication aids should be ready when the training courses begin. The trainees should be able to take their first kit of communication aids away with them after the course.

Within the rural village community itself, it is important to identify numbers of "audiences" who need to be reached and helped to undertake behavioral and attitudinal changes. There is, in reality, no such thing as "the rural masses" or "the villagers" as such. Communities consist of different age-groups, occupations, levels of education, roles of leadership or authority, types of influence within the family, and so on. Analysis will identify that many of the behavioral objectives have very specific audiences and need messages suitable for those audiences.

An example is a nutrition program for pregnant mothers. The pregnant mother is a highly sensitive audience: the technical content of nutrition messages and the way they are presented are very specific to her. How she pictures the child in her womb, what universe of values and suppositions she lives in during pregnancy, what food means in those months, what she understands about the growth of the child in the womb--all this is necessarily involved in determining her behavioral objectives in an ANP. So is her relationship with the people in her family and in the village who are likely to influence her dietary behavior and hence her response to ANP messages.

To plan for an ANP role in relation to behavioral change in pregnant mothers, and thus for communication, the following are suggested as some crucial questions that need to be answered:

What precisely are the priority or critical nutritional deficiencies among pregnant mothers, in the project areas? Do these differ significantly in different project areas?

Which specific foods are proposed to remedy these deficiencies? Are these nutrients grown in the area, but not (or not adequately) consumed?

Could these nutrients be grown, and is this judged the best economic and administrative way to achieve their consumption by pregnant women, or are pills, etc. more viable? Or should specific combinations be recommended?

What are the existing behavioral and attitudinal patterns for pregnant women in each project area, in relation to intended ANP nutrient inputs? Will such new nutrients encounter deep constraints and objections? Why? What will these existing counter-nutrient reactions be?

Who influences pregnant village mothers about their food habits? What is known about their views, and the reasons for their views? Do these influentials respond to the views of other, identifiable people in a village (religious, lay, other leaders)?

Putting the answers to the above together, which identifiable types of nutrients, and means of providing them emerge as the most feasible to try for through an ANP?

For the selected nutrients, which specific behavioral and attitudinal changes must be sought among pregnant women and those who influence them?

In each project area, what other development innovations are being advanced with mothers? What behavioral changes are development agents (health, home economics, family planning, etc.) trying to achieve? Will the addition of these nutrition-behavioral inputs cause confusion? What integration of all such messages will be needed? How should this be programmed? When, where, and by whom will any other change agents be retrained, if necessary, to insure message-sense or compatibility?

What groups of ideas and information are most likely to help pregnant mothers start to consider and, it is hoped, to adopt the selected new habits?

Should these messages be positive ("If you eat vegetable X your baby may have weak eyes and might go blind")? How does the ANP authority plan to research and test such crucial questions?

What groups of ideas and information are most likely to make an impact on those who traditionally influence pregnant mothers? How should these be presented? If influentials have a powerful role over the diet of pregnant mothers, should additional messages be conveyed "anticipating" what the influentials will say about the proposed new food habits?

For each project area (in case there may be differences), what language, what specific ways and words for conveying these messages should be used?

Who, as specifically as possible, within the village and among government change agents available, should be the transmitters of these groups of messages?

For the chosen interpersonal and local-group transmitters, what communication aids (visual-printed, audio-visual, food-group models, etc.) will be needed, with which messages in them, and how presented? Who will design and produce these aids? What budget?

Where, and by whom, will these transmitter-agents be trained for these roles? What training aids needed? Who will make them? What budget?

Who will supervise their efforts, and reinforce and help them? Who will evaluate their work, and to whom will this evaluation be sent? How will on-going evaluation be used to modify the ongoing communication program?

What supporting/reinforcing roles can other people or communication media (i.e., radio, periodic mobile unit shows, etc.) play?

What communications software will be needed for these supporting media? What should the messages be? How can the ANP insure that the interpersonal communicators will participate in programming these supporting media and know what and when such media will be communicating? Who will design and produce the software? What budget? Who will supervise and coordinate for these media, and how will their impact be evaluated?

The above list of key questions needing definitive answers will need to be applied --with relevant modifications and additions --for each child age groups whose nutritional deficiency is selected as a priority objective of the ANP. Several guideline-factors may be mentioned in making up the list of questions for other age groups:

Behavior/attitude factors for improved nutrition for (a) the child and mother during breast-feeding; (b) the child during weaning; (c) the post-weaning toddler; will need to be researched and assembled carefully. It is quite possible that food habits and the traditional attitudes about them in respect, say, to the mother's diet during breast-feeding, may bear little or no logical relation to the habits and attitudes in the same community regarding food consumption during pregnancy.

It may be important to examine together the behavior "models" assembled for these several stages in the life-cycle of the child and its mother, to see how messages to the mother (and influentials) should be interrelated. It may be vital to insure that nutrition messages conveyed to the mother for pregnancy are not confusing in relation, e.g., to toddlers.

Assessing nutrition education beyond pregnancy care is needed not only in determining the influentials, but precisely who is usually involved in care of the child after birth. The mother's (and father's) daily activity patterns will need study, to determine for the actual project areas at what times of day the mother may be away from the home working, and the child in a given target age group left in the care of, say, an older sister, perhaps supervised by a grandmother. In short, the audiences for nutrition communication after pregnancy may be multiple.

It is urged that special attention be given to this widespread factor of the older sister who cares for small children in large or high-frequency-birth families. These young girls cannot be reached through the ordinary ANP inputs in the local school system. In many rural societies, girls never enter primary school. Even if older family members can be reached successfully with nutrition mesages, they may not convey them accurately to the young girl.

Adequate pre-program behavioral research along the above lines may well lead the communication planner to identify needs in the total ANP that may otherwise be missed. For example, because successful communication in development has to work with assured material inputs, the communication planner may need to recommend a whole activity as essential both for assured nutrition inputs and for a minimal venue for communication to those caring for toddlers.

This is to re-emphasize the fundamental importance of real, comprehensive behavioral research in the project areas.

In the key questions in the earlier section, reference is made to language and idiom. In terms of communication support, this cannot be overemphasized. With the best of intentions, development project planners in cities may adopt terminology for a program that makes sense to them, but no sense at all to the intended beneficiaries and community implementers. For instance, what does "applied" in the title "Applied Nutrition Program or Project" mean to any layperson?

Any complete project which involves a community should have a name that is positive and dynamic in meaning to that community and attempt to find a name, in the idiom of the community, that will communicate at least positive change connotations.

The planners of a community-level development project in nutrition should also make no assumptions about any of the detailed terminology which trained project staff will start to use. Within a few years, hundreds upon hundreds of trained ANP change agents may be showering rural villagers with words that are totally bewildering unless the vocabulary is pre-tested.

Communication planners for an ANP must know, right from the beginning, what the wise and properly checked terminology is going to be. The words and phrases used by the designers and producers of all means of communication must be coordinated.

Take a selected list of concepts needing language into sample villages and test-research their meaning as received. What language can best establish a positive connection between eating, food, and health? What words are best for the specific foods and their sources that the nutritionists wish to get consumed? Which should be used in discussing a child in the womb, and the whole concept of how it receives nourishment from the mother?

How can a language be developed that will convey the idea that there is quality of a certain order, not just quantity, in a mother's breast milk, and that the quality is determined by what she eats? How to convey that the quality and combination of food eaten may affect the child's mental alertness (or worse)?

Care must be taken to avoid patronizing ideas. In many cases, assumptions by planners that "the villagers will never understand such-and-such" result in their failing to find and use village-rooted wise people who may be able to give them an entirely viable way of communicating a difficult concept.

The support-communication personnel for an ANP will need the results of good project-area research into the use of visuals of malnutrition. Care must be taken not to use overdramatic, extreme examples; if such cases are rare in a given project area, the examples will lack credibility. All of the above applies with equal importance to training aids, for training of project staff and community leaders.

There is evidence from some operational research and evaluation that negative/warning messages to pregnant mothers may be more effective than positive ones; but this may not necessarily apply in every society. No automatic assumptions should be made that either (or even both) positive and negative messages are viable.

The communication planner must assemble all available knowledge about the media-perception and media-use patterns of people in project areas. Are people accustomed to films, and if so what kinds of films, screened where? What is the experience of using posters and what kinds should they be? Are radio sets widely available, how are they used, and has their impact been evaluated?

In the foregoing, stress has been laid on communication aids that can be used by project personnel, "close in", for group and interpersonal support-communication of an ANP. This is not to neglect the importance of mass media. However, broadly:

Radio can be a powerful help in creating awareness of a problem and of the possibility of change to meet that problem;

The ground-level change agents can seek to strengthen that awareness, answer the questions it may generate, and provide audio-visual examples of feasible action by a community to achieve the needed development;

When this process has begun to have effect, radio can be used--if properly programmed --to reinforce consideration and even adoption of the needed innovations. Spot-broadcast reminders about nutrition can be very useful at this point.

If it is possible to take this multi-media approach further, the ground-level change agents may be able to develop (or use already established) organized listening groups, with subsequent discussion as an objective, and ideally with taped feedback provided by change agents equipped with portable recorders.

Ultimately, radio can begin to deliver complete nutrition communication, integrated with other community-level development communication, if possible.

But ground-level communication is vital in programs like an ANP, and funds must be spent on proper training aids and then village-level communication aids, if radio is to play its appropriate part.

Before being able to assemble a plan for all this, the communication planner should make a very thorough resource inventory. This must include inventory of:

Available personnel for design, production, distribution, research/evaluation, and support-communication management;

Available production and distribution equipment, and administrative actions needed to put it at the disposal of an ANP;

Already-existing, available budgets in the cooperating ministries for ANP-support communication, and administrative actions needed to tap these budgets.

Only after this inventory has been completed, and the training and support-communication program planned, can the total budget needed be assembled, including new national and international assistance.

A critical question is where to locate which resources, for which purposes, as between the center, the provinces or states, and levels closer to the villages. The following guidelines may be mentioned:

In any large-scale, geographically diffused ANP, there must be a clear resource-system for training at different levels, and a well-planned training program, headed by a team of skilled personnel. Over a period of only a few years, thousands of people will have to be trained for a large-scale ANP. Without a uniform design and a team of people charged with this work, training will be haphazard, course content will not be uniform, vital behavioral inputs will be impossible to program, and there will be little chance of

good evaluation and feedback.

In foregoing paragraphs, key linkages between communication research and action, and training, have been cited. It is vital to look upon training, and both mass and group/interpersonal communication support, as one continuous and integrated process. Experience indicates that the best way of achieving this is bringing together the training team and the communication team in one unit (with whatever field branches may be needed).

Because ANP is multi-sectoral, existing resources for both training and communication may be divided between several ministries and their sub-central divisions. These must be coordinated, or the program may break down. If there is one coordinating ministry, the teams described above should be set up in that ministry with coordinating staff down in the infrastructure at least to provincial or state level.

Finally, the innovation messages of an Applied Nutrition Program probably will reach audiences that are receiving a great many other development-innovation messages at the same time. For instance, farmers may be receiving advice about high-yield varieties that may wholly conflict, in message received, with advice about nutritious food production which the ANP needs to disseminate. Other ministries may be urging community actions that request voluntary community labor on various public works, when the ANP thrust may be for pure water sources as the priority.

Development messages fighting each other raise questions much wider than those of the support-communication planner. But it must be emphasized that the nutrition-support communication planner is bound to seek out these potential clashes, and try to get them resolved if his or her work is to have a chance of success.

UMBANDA RELIGION AS A POTENTIAL DELIVERY SYSTEM FOR NUTRITION INFORMATION IN BRAZIL

Maritta Koch-Weser*

This paper deals with Umbanda in Brazil as an example of a popular religious movement as a possible source of cooperation and a means of communication in a nutrition or related program.

Umbanda: character and organization

Although on the verge of establishing itself as a nationwide "church", Umbanda now is a non-dogmatic and fast-growing popular movement. In fact, it is presently one of the world's most dynamic religious movements. Umbanda is an urban phenomenon, attracting most of its new adherents from rural-urban migrants. More than for any other reason, people join Umbanda initially for advice on health problems and treatment of emotional disorders. During Umbanda sessions, they receive individual counseling, including practical hints, such as which hospital to turn to, together with magic recipes, and ritualistic-symbolic procedures which are believed to be beneficial.

Umbanda is presently trying to step out of the shadow of the Catholic church and to disassociate itself from what is now

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perceived as "savage" African tradition, namely animal sacrifices. Followers from the upper classes lend previously unknown prestige to this type of religion. The more educated like to think of Umbanda not so much as a religion, but rather as a law, which moves spiritism into the field of natural sciences and supposedly far beyond any rational doubts.

Umbanda followers may well coincide with the target group of a nutrition program, and its educationally distinct leadership (which ranges from retired generals to medical doctors and teachers) will probably be open to rational arguments and so be able to collaborate effectively.

Some superstitions obviously correspond to religious traditions and myths; others have grown over time, sanctioning habits (e.g., good) and social conditions. Food, by tradition, is an important topic within Umbanda ritual: in the Afro-Brazilian cults each god ("orixa") requires a certain set of offerings, i.e., animal sacrifices, foods, and herbs. Persons devoted to these gods have to observe corresponding restrictions. The custom of animal sacrifice is diminishing; in numerous cult centers, animal sacrifices are reduced to one or few occasions per year, or discontinued altogether. This development provides an excellent example for the justification of change by reinterpretation within the same pattern of thought.

Ritual food taboos, where they are maintained, refer almost exclusively to certain meats--those that are agreeable to a certain "orixa". Abstention from certain foods and fasting, together with ritual baths and herb preparations, play a part in initiation rites and stages prior to trance in general. New products which are not related to traditions of long standing, such as Coca-Cola, beer, or "Guarana" (a soft drink) are offered to the gods in addition to traditional foods and drinks. This field might be worthwhile exploring in more detail, in order to establish common trends in the acceptance of innovative foods.

Comparing ritual taboos to the diet of Brazilians in general, it turns out that none of the items most missing in the traditional diets of the poor go along with any of the ritual concepts - eggs, green vegetables, fruit, or milk products have no role assigned to them in the cult. Thus, Umbandistas share the general food concepts of the Brazilian poor, which may be considered as an advantage, in the context of a possible nutrition program.

Following is a brief outline of the general food concepts:

The native hunting and gathering Indians have had relatively limited, though fundamental, impact on their country's diet, contributing the use of manioc and corn. Some remainders of Portuguese diet in Brazil can be seen in the general preference for greasy foods, in the use of sugar and salt - unknown in pre-colonial Brazil - and in the popularity of sweet desserts rich in eggs. The Africans made substantial contributions to the Brazilian kitchen. While their regular meals on the plantations consisted of no more than manioc-flour, beans, rice, bacon, and occasionally bananas or oranges, they supplemented their food, wherever possible, by additional subsistence farming products, as well as those from hunting and fishing. Eggs, one of the most conventional foods in Brazil, were used by African slaves for curative purposes only. In general, most lower-class persons (5 out of 10 Brazilians) maintain that eggs have ill effects for certain organs of the body (e.g., the skin, the liver, or the teeth), or just in general.

Food beliefs and concepts vary regionally so much that it is difficult to generalize. Most Brazilian food taboos and habits refer to small children and the elderly. Both groups are forbidden certain meats, eggs, seafood, fruit, cheese, or beans. This contributes heavily to chronic malnutrition, especially among infants. If Brazilians are sick, the popular diagnosis is that they have liver problems. Accordingly, unpopular foods are said to be bad for the liver. Fruits and vegetables--also designated respectively

as dog, pig, monkey, or slave food--and milk are supposed to cause liver problems, while no such doubts exist concerning the fat bean dishes and "cachaça."

Such concepts among the poorer and less educated classes cannot be attributed to ignorance alone: prices often have a prohibitive effect, which excludes changes in the basic diet. In former times, rumors concerning alleged ill effects of scarce food items may have been spread deliberately by the ruling class, but they have a different function today: poor parents cling to them as a welcome excuse in cases when they cannot afford certain foods their children might want. Rather than confronting them with the hard economic facts, those parents warm up the old, gruesome stories, explaining why one should not have what one cannot have.

A strategy for the involvement of Umbanda in nutrition programs

Points which recommend Umbanda as a delivery system for nutrition information are:

Any nutrition program in Brazil would inevitably have to cope with the two factors of "cultura popular" and religion. Even if an intended program conflicts with only a single aspect of the religio-cultural complex, it may arouse general opposition to the entire program. However, religious leaders and agents could use their authority to reinterpret customs and rules in a way favoring intended innovations. They combine the advantages of social and religious prestige, and of excellent channels of formal and informal communication within their community.

In the cities, the rural-urban migrants who are Umbanda followers are most likely among the social target groups of all possible nutrition programs, because they are the neediest.

Umbanda's strongest following is among women, which makes it especially attractive for any program that stresses mother and child care. The participation of young girls should be further investigated; studies carried out by the Catholic church in Brazil show that religious participation is highest among women before marriage. Therefore, premarriage age would appear to be important for any educational efforts within a religious community.

Contrasting popular traditions from different parts of the country flow together in Umbanda. Their confrontation may be helpful in eliminating certain beliefs or in reinterpreting them. By joining an institutionally new movement like Umbanda, its followers have demonstrated some openness towards innovations. In addition, Umbanda is one of the few functioning networks within poor and fast-growing slums. It offers social services, such as health-care centers and orphanages. It combines social and educational ideals with a good communication network. Gossip, which extends beyond the cult group itself, may be valuable. Umbanda runs its own radio-programs, has its own journals on the newsstands, publishes innumerable books, and could well distribute posters in its terreiros. Proof of the effectiveness of their propaganda is not only the Umbanda movement's own growth, but the support by Umbandans of certain candidates.

As a first step, any nutrition program should be exposed to the following:
(a) Does it directly contradict certain food taboos?
(b) Does it refer to food habits common among cult followers?
(c) Does its target group overlap with Umbanda followership?
(d) Does the number of the Umbanda followers in the given community make it worthwhile to address them specifically?
(e) Does the inclusion of Umbanda and/or other cults seem to be a basically noncontroversial step in the eyes of the entire community, thus promising to improve, rather than to hamper, the program by its collaboration?
(f) Are the communication media which the religious group offers of interest for the program?

If such a general examination leads to the expectation that the religious group could be successfully included, the acceptance of the intended nutrition program should be tested with a sample of Umbanda groups in the given community or communities. Innovations must start from the top, so the cooperation of Umbanda leaders, the confederations' prestige, and, primarily, their files, are indispensable.

The most representative way to test the program would be to interview Umbanda cult group leaders, who tend to determine and represent the value system of their cult group. Some of the cult leaders have a good educational background; most do not. This implies that they are not accustomed to answer questions specifically and to the point, which makes interviews very time-consuming; therefore, only the most relevant questions should be asked.

After carefully explaining the purpose and the advantages of the intended nutrition program to the interviewed leader, the following and other possible questions should be posed:
(a) Which are the foremost nutritional needs within the radius of the cult group, which are therefore the most valuable components of the program?
(b) Where would he or she anticipate problems concerning the program's acceptance?
(c) Which measures might he disagree with personally?
(d) How would he argue if he had to promote the program?
(e) In what practical ways could he support it (e.g., would he be willing to teach, put up posters in his terreiro, invite a lecturer on the program, or serve as a distribution point for the program)?

A small sample of this type within each given community will probably be sufficient, since Umbanda concepts tend to vary strongly in detail, but little in substance. After thus establishing the local mainstreams and trends, the next step would be a proposal addressed to the local or regional Umbanda confederation, concerning the possibilities of their cooperation in the program. For tactical reasons, the proposal should come in the form of a flexible petition. and not as a definite concept; Umbandistas enjoy respect and courtship.

The program should distinguish clearly which of its objectives it wants to reach through the communication channels at Umbanda's disposition. The more complex messages should preferably be brought forth in radio programs, in which one can hope for correct presentation; the ideas to be promoted by the cult leaders should be simple and clear. The messages promoted at the terreiro level should be accompanied by poster messages or, if the program entails such measures, by the distribution of certain advertised items in the course of the weekly public sessions. The Umbanda journals are read primarily by cult leaders, and only subsequently by those in the terreiro who are literate and interested. Any messages in these journals should therefore address the more educated Umbandistas.

Finally, a nutrition program's success and acceptance within Umbanda should be relatively easy to assess at certain intervals and over a longer period of time within some of the fairly stable Umbanda terreiro groups.

CASE HISTORIES IN MASS COMMUNICATION

I. BALAHAR - THE INDIAN CHILDREN'S FOOD (A Case Study in Communications to Promote Better Nutrition) by Sylvester daCunha*

A few years ago, a severe famine overtook Bihar, a state in Eastern India with a population of around 50 million. It was feared that some three to four million rural poor were at risk of starvation. The Government of India, as well as international aid and voluntary agencies rushed in to avert what threatened to be a catastrophe of monumental proportions. Toward the end of the famine, the Government introduced a mixture called "Balahar", which translated means "children's food".

Objective

The US Agency for International Development (USAID) commissioned an advertising agency to design a communications campaign to create awareness of and demand for Balahar after famine conditions had disappeared. The intention was to so package, price, and retail Balahar as to win continuing acceptance among those who were in nutritional need of it.

Background Research

Prior to designing messages and materials, the implementing agency undertook two broad categories of research. First was a field study by a team including a sociologist, a social worker, a communications expert and a graphic specialist, who toured the area, met social leaders, school authorities, government field workers, etc. They reviewed some of the conditions relevant to the task and studied the media possibilities. There was no organized media like radio, TV, newspapers, etc., so it was necessary to identify new channels of communication in the rural context.

The second part of the research involved the questionnaire - and - interview method, and was conducted among teachers, parents, and children. The survey sought to throw light on:

1. Foods eaten by children at different meals;
2. Awareness among children, parents, and teachers of the various free foods supplied at schools;
3. Children's preferences among the free foods being served, including Balahar;
4. Awareness of the nutritional values of free foods, including Balahar;
5. Awareness among children, parents, and teachers of the supply sources of the free foods.

Findings

The important findings were that school teachers were the most important change agents in respect to changing dietary practices; the children came next; parents were the least potent force, as they were, in the main, illiterate. The experts came to the conclusion that, if a certain brand of food is distributed as a welfare commodity during times of distress, its continuance in a commercial form after the emergency should be under a different brand name, so that it not suffer from adverse associations with the past.

Dinner was comparatively the largest meal of the day. Balahar supplementation therefore appeared most desirable with the morning or midday meal. This endorsed schools as a delivery system.

The name Balahar was virtually unknown, as no attempt was made to create brand identity. This was an advantage, since there was little knowledge of the nutritive values of Balahar, and it was regarded merely as an emergency food distributed to combat famine. This ignorance applied

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*Managing Director, daCunha Pillai Associates Private, Ltd. Elysium Mansion, Walton Road, Bombay 1, India.

to both teachers and parents, and pointed to the need for education. Children preferred the taste of Balahar to the other aid foods, but teachers commented that the food was not a ready-to-eat item. The need for kitchen arrangements to serve Balahar was seen as a constraint to its widespread use. A precooked form (as a biscuit or a pancake) was recommended by teachers.

Message Material and Media

In designing the communication programs, three target groups were identified: teachers, children, and parents. The overall campaign consisted of two main components. One was that of creating awareness; the second of imparting education. The awareness campaign included posters, hoardings, messages over mobile loudspeaker vans, and films for touring government audio-visual jeeps. The basic campaign slogan was "Balahar strengthens a child's body and improves the mind". This theme was built around a small boy flexing his muscles and a special logotype for the name Balahar was designed in the local languages. The presentation of these three elements was uniform in all the visual media, so that a uniform and integrated impression could be created in the shortest possible time throughout the community.

The teacher communication was rational and informative, supporting the claims of Balahar to deliver nutrition of a high order. Children were appealed in terms of the taste factor and as a "stronger body and better brain promise". Parents were promised that Balahar offered their children "a bright and healthy future".

Outdoor media, such as buses and cycle-drawn rickshaws, were to carry the Balahar message. Match boxes, which are effective in the remote countryside, would display a Balahar advertisement. Religious calendars were designed for distribution and display in homes. Linking nutrition with religion and folklore helped greatly to strengthen Balahar's message.

The channels for reaching the teachers were a simple illustrated manual on nutrition, which was suggested to be put in the course of special organized seminars in each area. This was reinforced by flip charts, take-away leaflets, and other audio-visual aids that the teacher could use in the school. Some of the lessons in textbooks were rewritten to include education on important aspects of food and nutrition. Sample lessons were devised for submission to the education authorities. Posters were designed for display at schools. The Balahar message on each school exercise book, as well as slates and blotting paper, were innovative media items. A Balahar physical fitness contest was recommended for each school.

The material designed for children was simple, take-home leaflets handed to them by the school teacher aimed at eventually reaching the parents. Although a high proportion of parents was illiterate, they would be impressed by the colorful material on Balahar, and the children were instructed to read the information to their parents, thus securing greater involvement within the home.

Other change agents, such as social workers, local government officials, and prominent leaders of the rural society were to be addressed through periodic meetings conducted by the Health Department to highlight the importance of improving health standards and stressing the role Balahar would play to this end.

In each of the rural schools, a seven-member committee ("Guru-goshti") existed and exerted an important influence on the management of the school. Direct-mail materials were pretested for comprehension among selected members of the target audience, and necessary changes were effected to remove misunderstandings, doubts, and misinterpretations.

Conclusion

The Balahar communications plan was intended as a possible format for a nutrition-

education campaign designed to support the commercial sale of a subsidized nutritionally balanced food.

The fact that it was not implemented underlines the reality that once an emergency is over on the food front, concern about nutrition tends to take a back seat in national priorities.

II. THE MODERN BREAD STORY (A Case from a Developing Country - India) by Sylvester daCunha

This note deals with the case of Modern Bread, an enriched white loaf introduced in the mid-sixties in India. Factors such as taste, tradition, or lack of money combine to make the Indian home difficult for new, improved products to break into. Let us consider Modern Bread, a product of Modern Bakeries Ltd. - a Government of India Corporation - made in a chain of bakeries constructed with the help of the Australian and Canadian governments. A loaf of lysine-fortified Modern Bread provides 100% of a person's daily vitamin A and thiamine requirements, 75% of iron and 50% of riboflavin and niacin, without affecting its taste.

However, Modern Bread ran up against religious bias. Hindu vegetarians, and there are a vast number of them, generally do not eat bread. Historically, bread came in with the British, and it was only the meat-eaters, several million of them, who accepted this food. The vegetarians stuck to their traditional, home-made, unleavened bread. Why, then, trouble to produce a better bread that no one wanted? Although a minority, there are millions who eat bread, and Modern was only one of an array of fortification projects contemplated by the Government. Modern happened to be the first.

On the domestic front, home-made unleavened breads take various forms and differ from place to place. In the North, a favorite is "paratha", a flat wheat pancake fried in oil. On the West Coast, "puri" is deep-fried and the top crust rises leaving a hollow inside. Further down the West Coast, the "chapatti", is dried-toasted, flat, and thin. South Indians must have their "idli", a steamed rice product, cake-shaped. In sum, Modern Bread had to face competition from nutritionally inferior white loaves and home-made breads. Modern Bakeries Ltd. adopted a two-stage strategy.

Stage I was to win over existing eaters of white bread with the argument that in Modern Bread they were getting a better loaf for their money. Stage II was to convert the unleavened-bread group. A better-food argument can make sense to the hungry on the ground that it is cheap; to good livers on grounds of taste; to status-seekers on grounds of prestige; and to the infirm on grounds of health. The Modern Bread strategists chose health. Some may argue that this was ill-considered, since consumers do not really expect to get healthier by eating bread. However, health was the angle chosen and, in India, it was proved to be a good taking-off point.

Modern Bread had a paper wrapping when it was launched early in 1968. Its design belonged not so much in a larder, as in a medicine cabinet. The planners were too involved in the technical and production problems to give much thought to packaging, styling, image, and consumer appeal. By the time the communication people were brought in, the wrapper had already been approved at the highest level. It was too late to change it before a health-platform advertising campaign launched Modern Bread. The advertising appeared in a multiplicity of Indian languages over a wide range of media.

The major point this advertising made was that the bread was fortified with lysine, "a unique ingredient that multiplies the protein value of food". This bit of scientific jargon probably sailed over the heads of consumers, although they did get the message that it was "a 100% vegetarian food". The continuing slogan in this campaign was "Good for both, health and growth". A young boy was used as an advertising symbol. In all the advertising, lysine got star-billing. The medical-type advertising

was not entirely without its rewards. It helped to lift Modern Bread out of the "Muslim-bakery" context and present it as a secular loaf made by a welfare Government with the latest scientific and technical know-how. At the end of the first year in December 1968, Modern Bread sales stood at around two million loaves a month, a relatively modest figure for India. The project as a whole was still to break even financially.

The beginning of 1969 saw a shift in strategy, i.e., Stage II. This strategy sought to enlarge Modern's share of the white-bread market and, at the same time, to reach out more aggressively to non-eaters. To this end, it was decided to switch the story from health to taste. A new series of five, special-flavor breads was taste-tested.

There was Masala Bread (masala is the Hindi name for "spice", very popular with the Indian palate), Fruit Bread, Sweet Bread (a sweeter version of the White Bread), Brown Bread, and Milk Bread. These breads were not fortified in any way. They were intended to change the image from a health food to a taste item; second, to get at a new section of the market--that occupied by buns, pastries, cakes, etc. The new varieties, it was hoped, would give Modern White Bread a foot in the door.

The old wrapper came under review. About 15 new designs were tested among households for their ability to project a tasty, yet health-giving bread. Reference to lysine was dropped from the wrapper. One design was finally voted the most successful. It was more modern and colorful than its predecessor, stood out better on the shop shelf, was clearly identifiable. It was economical to print in just two colors, and its basic design could easily be adapted for all taste varieties. The new advertising multi-media campaign used newspapers, magazines, cinema, radio, shop display, etc. The major emphasis was on taste, and only incidentally were protein and nutrition referred to.

A few months later, a steady rise in white-bread sales was recorded hand in hand with that of the varieties. Still later, the varieties took a downward dip, but the sales increase of the fortified white bread continued. As post-research showed, non-bread eaters sampled the varieties and, having crossed the brand line, ventured still further and tried the white loaf.

Modern Bread promotion has undoubtedly brought about fundamental changes in bread-eating habits. Vendors are selling buttered slices as a curbside snack. Bread, a dreary staple in Western countries, is acquiring the image of a tasty tidbit among a whole new section of our public who are eating it for the first time. Happily, Modern Bakeries Ltd. is now financially viable.

In conclusion:
1. The marketing people must be involved at the earliest stage of planning a new product;
2. The consumer must be studied and the product positioned in relation to his needs;
3. Educate before selling; precondition the market favorably toward a new product before it is launched;
4. Taste is often a more tempting bait than promises of better health.

III. A SUMMARY OF THE MASS MEDIA NUTRITION EDUCATION PROJECT IN ECUADOR* (1974 - 1975) by Richard K. Manoff

A radio-based mass-media nutrition education program began broadcasting in two regions of Ecuador during February 1974. Advertising techniques were used to promote health and nutrition practices throughout the coastal province of Manabi and the Andean region, including Tabacundo and extending through the southern part of Imbabura Province.

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*Conducted by Manoff International, Inc., 845 Third Avenue, New York, N.Y. and the Government of Ecuador. The project was sponsored by USAID. The above report represents an abridged edited version of the Summary.

The specific messages were developed with the cooperation of Ecuadorian nutritionists. Nutrition, health service, and government agencies reviewed the messages for correctness of the advice and conformity to established directives. Then the messages were tested among people in the respective target audiences for coherence, believability, and persuasiveness. The revised messages were retested before being produced in final form.

The Purposes of the Project

Education: Educate specific segments of the Ecuadorian population as to the viable solutions to specific priority nutrition problems.

Motivation: Motivate those segments to take steps to improve their nutritional habits.

Development of a working model for use in other developing countries: The adoption of commercial campaign techniques to social programs and testing them, was expected to provide a model of the mass media reach and frequency education techniques for other countries to emulate.

Cooperating Institutions

In the beginning, the project was attached to the Ecuadorian National Institute of Nutrition (INNE). Less than halfway through the project implementation, the responsibilities for local administration of the project shifted to the Nutrition Division in the Ministry of Health. However, the same people from the INNE participated in the implementation.

The media were expected to provide free broadcast time and several government agencies were to cooperate in the evaluation interviews, so it would have aided the project immensely had the project been associated not only with the Ministry of Health, but also with the National Planning Board and their national nutrition planning section, and to the Office of the Presidency. Throughout the project, the support and assistance of the local mission of AID were received. All the broadcast time was donated by station owners in two provinces, and some of the costs of the project were paid for by local business. Had there been a more sustained effort by the government or by some interested private group, the effect of the spots on generating private support would have been greater. Even before the broadcasts began, province-level nutrition committees were formed spontaneously in each area. However, the committees were not encouraged by the local health officials, and within a few months they were defunct.

Mass Media Situation

Radios serve as the primary means of communication for the rural areas. The government does not use television systematically to educate or inform the public. Television programming is composed primarily of imported materials, with little cultural relevance and no educational value.

Reasonably reliable data on radio and TV audiences exist only for the major cities. Cinema seems to be an effective means of reaching low-income people, but attendance is too infrequent to achieve impact through short messages. Newspaper readership is low, though access is relatively easy for short campaigns. Low literacy, high cost of purchase, and limited distribution keep readership low.

Results of media monitoring: During the first six months of broadcast, (up to about September, 1974), the messages were probably broadcast at a rate of 200 times daily. All messages received equal exposure.

Several stations stopped playing the spots during August, 1974, but during Christmas the frequency fell off generally and did not recover by the end of the campaign in April, 1975. These impressions of the frequency of broadcast, gathered during interviews, tend to confirm the drop in message awareness between the second and third wave of interviews.

The estimated cost of radio time was about $5,000 for two provinces that cover about one-fourth of the population. Costs were kept low in this estimate by excluding from the calculations the cost of buying time from the more expensive Quito stations. A national campaign using the same frequency would probably have required about $20,000 annually, if air time were purchased.

Use of television and other media: Television and cinema were the media for messages intended for urban audience. In addition, posters were produced and distributed that displayed the themes of the campaign. No records were kept of the poster distribution, nor was any evaluation made of the impact they had.

The Campaign

This project was the first attempt to use the reach and frequency technique to change diet and personal hygiene habits without a new product by using commercial advertising techniques. Messages were to be directed to two distinct radio market areas, one on the coast and another in the highlands, so the initial data review concentrated on the problems of urban slum dwellers (Guayaquil) and the rural families (Chimborazo Province).

The objectives of the campaign were:

Protein-Energy Malnutrition

Increase the frequency with which beans and other low-cost sources of protein are served.
Increase the knowledge about which foods are sources of protein.
Increase the knowledge about the function of protein in the body.

Early Departure from Breast-Feeding*

Increase the status of breast-feeding among low-income mothers.
Increase the recognition of the valuable attributes of breast milk as compared with other types of milk.
Increase the knowledge about the steps for preparing other kinds of milk.

Unsanitary Drinking Water

Increase the frequency of boiling drinking water for the family.
Increase the understanding that drinking unboiled water can result in illness.
Increase the number of families who consider their drinking water to be contaminated or not pure.
Increase the number of families that cover their drinking water supply.

Parasites, Diarrhea, and Other Intestinal Problems

Increase the frequency of adults and children who wash their hands after using the latrine, before eating or preparing food.
Increase use of soap when washing hands.
Increase the number of respondents who understand that washing hands may kill parasites and help avert illnesses.

Iodized Salt

Increase the frequency of purchasing iodized salt.
Increase the number of respondents who understand the cause of goiter.
Increase the number of respondents who understand that goiter is a serious illness.
Increase the number of respondents who know that iodized salt is sold only in a distinctive package.

Writing and creating: The scripts were written and revised after the copy testing and their technical accuracy and acceptability by the target group were under constant check. After approval at the highest level of the government, they were presented in a soap-opera format, with a young mother with a sick or underweight infant brought to see the doctor. At the end of each message INNE is credited as sponsor.

Production: All production was done in Ecuador, under the supervision of the

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*The message could not present an exact age until breast-feeding is recommended. The message simply stated that the mother should breast-feed as long as she is able. Under these circumstances, it is, of course, impossible to test for behavior change.

local representative of Manoff International, Inc. Professional voices were used for the radio spots and stock music. The Quechua versions also used professional voices and a popular Indian melody for background music. The radio spots were pressed into disks, one spot per side, and distributed to the stations.

Local equipment was used for cinema and TV spots with local personnel, except the chief producer, who was American. The film was finished in New York, as facilities for editing do not exist in Ecuador.

Media plan: It was not possible to guarantee scheduling spots at the prime listening times. When the program began, there was a blanket appeal to all the stations for their collaboration. This approach of accepting "run-of-station" scheduling, asking for a frequency of at least 10 spots per day per message per station, and including all stations regardless of their penetration of the target, is a far cry from the carefully calculated media plans of commercial clients. Nevertheless, this was the most appropriate approach for the first project in Ecuador.

In addition to the radio and limited use of television and cinema on the Coast, the INNE developed some posters and a logo for the campaign.

Research and monitoring activities: This project contemplated several data-gathering procedures that would assist in documenting the effect of the messages on the target group and the cost of production. In addition, field interviews were conducted to test the messages before they were broadcast.

THE RESEARCH DESIGN

The two objectives of research in developing and evaluating the mass media nutrition education program in Ecuador were to make sure that the messages were coherent, meaningful, and motivating, and to enable evaluation of the impact of the campaign on three different target audiences and modify the program where necessary during progress. The investigation undertaken to help interpret the effect was an awareness, attitude, and behavior tracking study, conducted in three waves, over a period of one and one-half years. It is the basis of all information on the effect of the program.

The target audience: The coastal Mestizos are the most affluent, educated and economically involved of the three target groups. The messages were recorded and broadcast in the coastal Spanish dialect.

The Sierra Mestizos, near the provinces of Imbabura and Pinchincha, were the second most affluent, educated, and economically involved. They did not have as good a health delivery as the coastal Mestizos, and they did not have sufficient access to television to warrant a television campaign; however, ownership of radios was widespread. Messages were broadcast to this group in the Sierra dialect of Spanish.

The indigenous, or Sierra Indians, also from the provinces of Imbabura and Pinchincha, were the least affluent, working for the most part as subsistence farmers. They were the least educated, had the least access to health care services, and radio ownership was low. Messages were broadcast in Quechua, the Indian tongue.

Awareness, attitude and behavior tracking study: The approach established benchmark measures of nutrition awareness, attitudes, and practices specifically related to the priority subjects of the campaign, and followed, at 6- and 12-month intervals, the changes in these attitudes and practices.

Specifically, the research objectives determined:
Awareness of the nutrition messages;
Awareness of the subjects and advice covered in the messages;
Attitudes toward the message recommendations;
Practices related to the messages;
Media habits;
Demographic profile.

It has been assumed that the mass media reach and frequency technique will affect social patterns in Ecuador in the same way it has been known to affect commercial consumption patterns and social patterns in other countries. However, the amount of time needed to change a social behavior in Ecuador was not known, nor were all the pitfalls that might work against a behavior change known.

Methodology

Three waves of interviewing were conducted. The first was implemented in February, 1974, before the messages went on air. It constituted the benchmark measure. Subsequent measures were made 6 to 12 months after the start of "advertising" (November, 1974, and May, 1975). For the first and second wave, a total of 1,200 personal interviews were completed. Samples were cut to minimal sizes in wave 3 (500 personal interviews), due to budget considerations.

In the study areas, sampling procedures, respondent selection, and interviews were conducted, using acceptable, scientific methods. Data processing was done by computer.

Summary of Findings

Comparison of Wave One and Wave Three: Throughout the analysis, wave-one and wave-three results are usually compared, because in most cases, although both results represent significant increases along the lines recommended by the messages, the wave-two levels are usually higher. It was felt that comparing figures obtained at similar times of the year and analyzing the less successful results would be less confusing. The primary reason for a falling off from wave two to wave three is a lowering of message exposure.

A second factor compounding the apparent decline was that, in wave three, the interviewers were more aggressive in probing and in eliminating answers which were not exactly true but which the respondents thought the interviewers wanted to hear.

Limitations:

(a) Compromising the questionnaire to meet the objections of the authorities. As a result, the final questionnaire did not contain several useful pieces of information and resulted in uneven probing by interviewers within the same wave and among the waves for the same questions.

(b) Limitations of the interviewers, due to inexperience and frequent turnover.

(c) Inherent deficiency of translations of questions, leading to loss of important shades of meaning.

(d) Noncompletion of supplementary research projects. Except for the iodized salt shipment, data collection deprived substantiation of the findings from the tracking study.

Message Penetration:

(a) Radio ownership or access to a working radio was the key to whether a person was aware of the message.

(b) The patterns of awareness and claimed behavior change in the data, as well as other factors, suggest that there was a letup in the number of times each message was aired between waves two and three and, therefore, a decline in message effectiveness from wave two to wave three.

(c) Message awareness tests indicated that the messages reached into a majority of the households in both provinces, achieving a penetration in 15 months that would be enviable in most commercial campaigns and certainly greater than conventional face-to-face educational campaigns.

(d) As a result of each message, the respondents improved their knowledge about the subjects in ways designed by the messages.

(e) The iodized salt message was the most successful in achieving behavior change.

Dramatic shifts were documented among both Mestizos and Indians in claiming to use iodized salt.

Accomplishment of the Project

1. This was the first closely studied effort to use modern marketing methods, not associated with a product but with changing food and personal hygiene behavior.

2. The project was carried out in Ecuador, using many of the local people to do the technical work, recording, filming, interviewing, etc.

3. Broadcasting was done within 6 months after beginning work, in spite of handicaps.

4. The interview schedule, including nearly 2,800 respondents, studied over an 18-month period, is the largest field interviewing program ever undertaken in rural Ecuador, and the largest opinion-research project undertaken in the country.

5. It was possible to document changes in behavior, knowledge, and attitude as a result of the messages.

6. A national social-service program can get access to the radio airways and can provide professionally produced materials without a large investment in studios, writers, directors, and all the other costs traditionally associated with radio programs.

7. It has been demonstrated that this technique can be used to educate rural mothers, farmers, and young people without an investment of thousands of dollars for studios, etc.

8. It has been shown that this model is appropriate for other countries and may achieve greater impact because of the lessons learned in Ecuador.

IV. THE TETU EXTENSION PILOT PROJECT, KENYA by Joseph Ascroft*

The Tetu Extension Pilot Project is one component of the multifaceted Kenya Special Rural Development Program (SRDP). Six relatively small areas of Kenya were designated for special experimentation with new strategies. One of these was the Tetu Division, located close to Mount Kenya, which has about 11,500 farm families with average holdings of about seven acres. The Tetu project began in June of 1970, was projected to continue for five years, and was handled by the Institute for Development Studies (IDS), University of Nairobi, using funds provided by the Kenya government.

The total direct costs for running the project was K 14200/(slightly more than $2000 U.S.) or K 65/11 (about $9) per project participant. These figures include an input loan to each participant which, if all repaid, would reduce the total cost to about K 3567/-($510) and the per capita cost to K 16.50 ($2.50).

Nature of the Project

The project was designed to develop and test strategies to accelerate the adoption of innovations for the physical, social and/or psychological welfare of rural farm families. The project focused on increasing agricultural output via farmer education in improved cultivation techniques and the adoption of improved seed varieties. A Kenya Agricultural Experimentation station had developed a variety of hybrid maize which increased yield per acre considerably and gave promise of making Tetu self-sufficient in maize production if substantial numbers of farmers switched to it. It was considered that substantially increasing per capita maize production would increase available staple food supplies and result in surplus production, which could be sold or bartered to gain other supplementary foods, thereby resulting in nutritional improvement.

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The Target Audience

In 1970, 31 per cent of the farmers were already growing hybrid maize. Included among these were early adopters and opinion leaders, though many of these still needed to improve their cultivation techniques and were not getting the maximum potential yield. Nevertheless, since the early adopters had already accepted the innovation, much of our diffusion work had already been done for us.

Among nonadopters, awareness of hybrid maize was high, but not the knowledge and skills of cultivation techniques to grow the seed successfully. Nonadopters, approximately 8000 farm families, therefore became the main target audience of the Tetu Project.

Subobjective

Tetu Division has within its borders a Farmer Training Center, which provides training courses in agriculture and nutrition, with board and lodging facilities for 100 people. It serves Tetu, along with many other divisions in the area. Participant observation in the classes at the Center and examination of the staff qualifications indicated satisfactory expertise in agriculture and nutrition, but little know-how in techniques of communication. In addition, the staff was youthful and employed an authoritarian form of address that proved unpalatable to the farmers and their wives, who were generally more than 40 years of age. Furthermore, too much detail was packed into their messages in too short a time without an attempt to de-jargonize their language or to interpret complex innovations. The field extension staff suffered the same drawbacks and, in addition, were not totally aware of what was being taught by the Center staff.

Thus, a subobjective was adopted to provide training in principles of communicating effectively with adult, largely preliterate audiences. Courses conducted by IDS communication specialists were arranged for both the agricultural and nutrition staff of the Farmer Training Center, even though the initial farmer training effort would concentrate more on agriculture than on nutrition.

The Media

As the project was restricted to one experimental division out of the 600 Divisions of Kenya, use of radio and television was precluded, except for publicizing that an SRDP project was scheduled to begin in Tetu Division. Instead, groups of approximately 50 farmers at a time were invited to the Center for a three-day forum of intensive training and discussion in techniques of cultivating hybrid maize.

In the first year of operation, 217 farmers attended the courses, which were timed to occur just before the rains came. Invitations were so designed that each group of 50 farmers was selected from the same sublocation. Because of their proximity, they were able to provide social support for each other and share experiences with their neighbors when they returned home to try out the innovation.

A two-step flow of information was thus designed in which the Farmer Training Center acted as a medium of mass dissemination and persuasion. To establish linkage between field and Center staff and to insure coordinated follow-up, each group of 50 participants was invited to the workshop along with the extension agent responsible for servicing its sublocation.

The Strategy Used

Growing hybrid maize requires more complex cultivation technology than does growing the traditional local variety; it ranges from early planting through land preparation, row-cropping, contouring, planting, spacing, fertilizing with superphosphates, weeding, thinning, top-dressing, dusting, to harvesting, storing, and marketing. Previously, only one three-hour lecture had been devoted to delivering these instructions to preliterate individuals.

Hence the communication strategy consisted of breaking down the overall complexities by "stretching" the duration of instruction to three days, focusing on each cultivation step one at a time. Thorough discussion was allowed for each step. Older discussion leaders were allowed to emerge from each group; young experts were called upon by discussion leaders as the need arose to provide detailed information and demonstration.

The goal was to take each group of 50 participants through the adoption stages of awareness, information, and evaluation of the innovation. To achieve this, a sort of balance sheet was drawn up, comparing the advantages and disadvantages of hybrid maize with those of local maize and allowing each participant to reach a decision to adopt or not adopt.

The intention was to get farmers to proceed to the trial stage, at which each participant would return home to try out the innovation on a small scale under the supervision of his extension worker. To minimize recall, farmers were put in groups of four to eight to carry out communal maize trials. This was so that a detail forgotten by one farmer would be remembered by another. Stenciled handouts were also provided, since some groups had members who could read and others could find somebody in their neighborhoods to read to them.

Grouping also reduced field follow-ups, since extension agents visited an average of six farmers at a time.

To overcome the farmers' problem of lack of funds, loans for inputs for one-half acre trial plots secured by eventual crop yields were issued, and the farmers themselves volunteered to supervise loan repayments. It was envisaged that repayments would be deposited in a revolving fund to be reissued as loans to subsequent adopters, such a fund to be administered eventually by the farmers themselves.

Starting in May, 1972, to the beginning of the rains in late June, 1972, seven workshops were held. Forum participants were selected, mainly by local officials in consultation with village subchiefs. Thirty per cent of the participants were women. Participation was voluntary for the farmers, but only 9 per cent declined the invitation. The high rate of acceptance was ascribed to the facts that the workshop was free and selected groups were from the same area.

Results and Evaluation

Only one of 217 invited participants decided not to proceed to the trial stage. Some participants accepted the loans, others used their own funds. Each group rotated from one group members' one-half acre trial plot to the next, repeating the procedures each time. The groups remained relatively intact throughout the trials, with members providing motivation for each other. More important, curious neighbors came to observe the trials and asked questions.

Interviews with 47 of the 217 participants, one from each group, were carried out at the end of the four-month trial period. An estimated average of 5.7 nonparticipants per participating farmers observed the planting process, i.e., more than 1000 (5.7 x 217) nonparticipants took interest in the trials. Furthermore, those interviewed claimed to know an average of 2.4 nonparticipants who bought seeds and inputs to conduct their own trials after observing the group trials. The positive effect of the Tetu experiment appeared to have considerable impact on the morale of Center and field extension staff, and they claimed to be under considerable pressure to organize more workshops in such areas as livestock management and nutrition.

However, at this point, systematic evaluation by the project experts and the evaluation team from the Institute for Development Studies ceased. The contracts of the communication experts (who initiated

the project) expired, and they departed the country to resume their normal pursuits. Nevertheless, it was the policy of the team of experts to help personnel to continue its management. A year later, the project continued to thrive, and Tetu Division was on its way to self-sufficiency in maize production.

Interpretation and Comments

The Tetu project demonstrates the feasibility of taking rural people from awareness to adoption of an innovation in a relatively short time by employing available channels of communication, which are not traditionally considered mass media. The secret of the apparent success of the project lies in careful planning and preparation, and in careful training of personnel in techniques of effective communication, curriculum development, and involvement of people. It took more than a year.

The next important step was the systematic selection of initial participants to maximize the diffusion effect to nonparticipants; that is, to involve people in deciding their own plans of action and so to influence their peers. The training of participants took only two months, and the field-trial period lasted four months, as it had to encompass an entire growing period.

If the type of project tested in Tetu worked for hybrid maize, there is no reason to believe that it would not also work for other agricultural products or for such areas of concern as nutrition improvement, which is allied in many ways to agriculture. Indeed, when the communication specialists left the project, these extensions were being planned by the local officials.

Furthermore, if the project worked in one division of Kenya, there seemed to be little reason to believe that it could not be replicated, with appropriate modifications, in other divisions throughout Kenya. Information trickling back to us from Kenya suggests that this is already underway in some areas.

Because the project was restricted to one small experimental area, use of the electronic mass media was precluded. However, if the project were to be replicated on a widespread basis, mass media (particularly radio) could be utilized to create awareness of the benefits and desirability of such a project. Moreover, movies can depict training procedures and trial groups at work to demonstrate the feasibility and pay-off of the type of project pioneered in Tetu Division.

V. THE INCAPARINA PROJECT by Susana J. Icaza*

Incaparina is a vegetable mixture with a nutritive value equivalent to that of cow's milk and with physical properties that make it an adequate food for infant feeding purposes. It has played a very important role in alleviating the protein lack in Guatemala, as well as other underdeveloped countries. The name is from harina, the Spanish word for flour, and means literally "INCAP flour". More technically, it is called INCAP Vegetable Mixture-9, developed in 1958.

Many factors had to be considered in the development of this new food: adequate quantities and quality of its components; ease of manufacture, transportation, marketing and preservation and low cost. It had to be nontoxic, and its nutritive value resistant to damage. It was necessary that its flavor and physical characteristics be in accordance with the dietary patterns of the population. It had to be easy to prepare and feed to children.

Data relating to all these factors were collected and at hand before work for the

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*Director, School of Nutrition, Center for Advanced Studies in Nutrition and Food Sciences (CESNA), University of San Carlos de Guatemala/Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Guatemala, C.A.

development of this new food was undertaken. The project was supported by funds from different sources, usually granted for a specific phase of the study. The new food obtained support from governmental authorities, particularly public health personnel. These workers recommended Incaparina to mothers of preschool-age children who attended the various clinics.

In its initial phase, acceptability tests were run in a community where an integrated health center was functioning adequately, and where acceptance of the health programs by the community members was evident. Demonstrations by a nutritionist to a group of mothers initiated the acceptability trials, and personnel were assigned to pay home visits and keep daily records for a period of three months on the amount of Incaparina ingested by every child in the home. These visits permitted collection of more data concerning local dietary patterns; types of dishes that could be prepared with Incaparina; possible frequency of its consumption and the members of the family who would consume it; their reaction to the product; the potential demand and the most desirable promotional approach.

The next step was an attempt to duplicate the first test in a group of representative communities. This was preceded by a nationwide, publicized press conference by the Ministry of Health, which informed the people about the benefits of this new product. A group of demonstrators was trained by a nutritionist and equipped with foods specially prepared with Incaparina. Information was given to community groups and the product was placed for sale in local stores. This promotion campaign was fully supported by the health center doctors and nurses in their regular work. Acceptance was high, and an initial period of inflated "curiosity" purchase started, followed by more stable sales.

The product then entered its third phase, introduction on a national scale. The new food was made available in local stores and efforts to reach the target audience began. Mass communication media and materials used for this purpose included posters, newspapers, radio and television commercials, food demonstrations, and group discussions. Messages were selected and tested prior to developing the materials and were used all over the country. Local demonstrations were given by health workers or food demonstrators in a combined effort to reach all the mother groups in each town where Incaparina was introduced. The demonstrations included a description of the properties of the product, a task in which demonstrators used flip charts and prepared a sample of Incaparina in atole (gruel) form, which was tasted by all who watched. Other demonstrations were conducted for religious groups, schools, and agricultural extension workers. If electricity was not available, panel vehicles were equipped with a generator and demonstrations were offered at night in the main plaza of the towns. A color film showing the effects of malnutrition was projected before or after the demonstration. In some instances, these activities were attended by as many as a thousand persons.

Evaluation studies were then designed. From 1959 to 1964 a study of three villages in which Incaparina was used as a food supplement in the diet of preschool-age children was conducted. It showed a drop in the overall incidence of diseases. Comparative surveys on diffusion of the innovation carried out in 1962 and 1968 in the same rural and suburban communities showed that the percentage of people who were not aware of the product's existence decreased from 69 per cent in 1962, to 16 per cent in 1968. During 1968, 64 per cent of the families in the sample who had children under one year of age, were feeding them Incaparina. This percentage was higher in families with children one to two years old (89 per cent), and reached 91 per cent in those whose children were two to five years of age. The percentage was lower among the older groups. Forty-three per cent of the people interviewed in 1968 said they had learned

about Incaparina from personal contacts, 35 per cent through the radio, and 14 per cent by non-personal contacts, especially at the local store. It is worth pointing out that, in 1962, those who learned about Incaparina through the radio represented only 14 per cent.

Another study carried out in 1968 concentrated on the marketing aspects of the product, and included families from all over the country. A total of 1,246 families were interviewed, and it was found that 84 per cent had some knowledge of Incaparina, a percentage that varied according to geographical area. The main sources of information on Incaparina were: radio, 33 per cent; local stores, 22 per cent; neighbors, 11 per cent, and health centers, 9 per cent. Only 29 per cent of the families with a monthly income under US $20 consumed Incaparina. This percentage rose to 61 per cent when the monthly income was from $21 to $100, and reached 76 per cent when income was higher.

Today, Incaparina continues to be sold in Guatemala, and the commercial enterprise in charge of its production had to enlarge its plant in order to satisfy the increasing demand. Unfortunately, the world economic crisis has made it necessary to increase the price, due to higher costs of the ingredients. This increase places Incaparina a little beyond the purchasing power of the people who need it most--the underpriviledged. However, in 1975, the Guatemalan National Health Department decided to subsidize the product, offering Incaparina at a lower price to those mothers who attend the well-baby clinics. Although still in its initial stage, the project may well be successful and beneficial.

VI. A PILOT STUDY ON THE FEASIBILITY OF USING MASS MEDIA FOR IMPARTING NUTRITION EDUCATION IN MAHARASHTRA, INDIA*

In India hardly any nutrition education is imparted through the normal education process in schools, colleges, and universities. What is urgently required is a "crash program" to educate very large numbers within a short span of time. The two basic alternatives available are person-to-person communication and mass communication. Because of the numbers involved, person-to-person communication will be prohibitive in cost and perhaps unmanageable administratively. Also, the effectiveness of person-to-person communication would vary considerably with the teacher quality.

On the other hand, mass communication has the advantage of being inexpensive, effective, persuasional, uniform and repetitive, and most importantly, is available in India.

A pilot mass communications effort was launched in the State of Maharashtra to evaluate its potential in imparting nutrition education to the different segments of the population.

The Objectives

The short- and long-range objectives of such a campaign were:

1. It must inform people about protein and its role in health and create an awareness of the problems which exist. The campaign must also inform them about balanced diets and desirable dietary practices.
2. It must motivate people to make the sacrifices necessary to improve their diets, perhaps to forego a visit to the cinema in order to give their child more milk. It must also alert people in positions of authority and responsibility to the dimensions of the problem and motivate them to take corrective measures without delay.
3. The campaign must lead to action on the part of those addressed. People must be persuaded to modify their diets, not in terms of expensive supplementary processed foods, but by balancing the diet with

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*Prepared by the PAG Secretariat. Based on the report kindly made available by the Protein Foods Association of India, 22, Bhulabhai Desai Road, Bombay 26, India.

ingredients that already are part of the family menu.
4. It must influence the State to appreciate the problem and help in its solution by rationalizing food laws, taxation, and the like.
5. It must create the atmosphere for the acceptance of low-cost nutritive foods, particularly those designed for the most vulnerable groups.

The Strategy

The operational strategy was:
1. To run a pilot operation in a single linguistic region, as the smallest economical unit for the use of mass media.
2. To use field research to evaluate the effect of the mass communications campaign on the exposed public.
3. To so design the operation as to apply this experience to other regions and, eventually, to the whole country.

The crux of the message was: (1) protein is a very important part of a healthy diet; (2) the right quantity and quality of protein is vital for well-being, particularly of children, both before and after birth.

The target groups for the campaign were those who may be termed the reachables and the persuadables. These had a nutrition problem, and some spare income. In concrete terms, these were people with a family income of at least Rs. 250 per month (US $35) living in towns with population of 20,000 and more.

In regard to the creative strategy, the campaign was woven around children, both because they are the most vulnerable nutritionally and because they have the greatest emotional motivational pull. The campaign particularly focused attention on the three most crucial stages of child development--pre-natal, weaning and preschool. The visuals and the sizes of advertisements were evolved bearing in mind the need to capture and hold the attention of the maximum number of uninitiated readers.

In the media strategy, press and cinema were used extensively. In the press, a total of 608 insertions were made in 48 periodicals and dailies in four languages, English, Hindi, Marathi, and Gujarati. Two 60-second films were screened in 139 cinemas, amounting to a total of 2950 cinema weeks. A slide exhibition was arranged for a period of three months in 26 cinemas showing Indian-language films.

Among outdoor media, 25 hoardings were put up in 11 towns outside the metropolitan area of Bombay. Out of these, 21 were displayed for one year and four for a period of six months. Three thousand information charts in three languages -- English, Hindi, and Marathi -- were distributed as poster material in hospitals, dispensaries, health centers, maternity centers, and clinics all over Maharashtra. The main effort was in the Indian-language media, as these had the maximum reach among the middle-income groups whom the campaign was primarily hoping to reach.

All these media carried a hidden offer of a free booklet entitled "Protein is Life", which provided more detailed information on nutrition in simple language. Eventually, more than 30,000 such booklets in all the four languages were mailed out in response to requests.

A 20-minute documentary film entitled, "A Horoscope for a Child", was released through the Films Division, Government of India, at about the same time. This was shown all over the country, and it served to intensify the pitch of the campaign in Maharashtra.

In addition, a public relations dimension was also incorporated in the campaign to influence opinion leaders. It embraced newspaper editors, textbook authorities, and so on, to pass on the nutrition message.

Cost

The total cost of the campaign was US $100,000: press, 48%; cinema, 24%; booklet, 6%; hoardings, 6%; other media 3%; research, 13%.

Research

The research study was carried out as three separate surveys:
(a) The benchmark survey, designated B, completed before the launching of the campaign, and intended to determine the levels of nutritional knowledge before the start of the operation.

(b) The mid-term evaluation, or M, was undertaken six months after the start of the campaign, and was mainly designed to "monitor" the campaign in terms of the creative effort, the sort of effect which it was having, and the research methodology. A smaller sample size was used in this survey.

(c) The final survey, F, carried out at the end of the one-year campaign, was designed to evaluate the campaign both in breadth and in depth.

Representation was given to all three regions of the state, viz. Western Maharashtra, Maharashtra, and Vidharba. While the three large cities were sampled in all the surveys, the selection of the eight other sampling points (two medium-size cities, three large towns and three small towns) was done wholly independently in each of the three surveys.

Sample sizes used

The benchmark survey was confined to mothers with children aged seven years or less, who were the target group of the entire campaign. However, during the next two surveys, it was felt useful to have the research cover the total adult population. Accordingly the mid-term and final surveys were designed to cover the total adult population and the group of mothers just described, who were now designated as Special Group Mothers.

The sample sizes used in the three surveys, and the estimated number of persons constituting the groups surveyed, are shown below:

	Numbers in Maharashtra	Number of families used in survey		
		Benchmark B	Mid-term M	Final F
Total adults	5.1 million	-	594	1566
Special Group Mothers (families)	0.71 million[a]	1610	339[b]	911[b]

[a] Representing 2.8 million persons of the total adult population.

[b] Households representing a subsample of the total sample; the actual respondents in the two cases were different, however, barring some overlapping.

Exposure to various media

A rough estimate was made of the degree of exposure of both groups to the various media, with the following results:

	Total adults, % exposure	Special Group Mothers, % exposure
Read newspapers/periodicals	72.8	59.5
Go to cinemas at least once a month	53.4	47.1
Listen to radio	48.0	70.3
Are not approachable by any of the above media	11.2	17.2

These figures obviously include overlapping.

Results in review

The field covered by the research study, which may be referred to as the research universe, related to all adults aged over 15 years living in 73 cities and towns of Maharashtra with populations of 20,000 and more, and belonging to families with a monthly family income of US $35 and above. This total adult population numbers 5.1 million.

The economic classification of the target population in terms of monthly family income was: US $35-70; US $70-140; above US $140.

Within this population, a special group was identified, comprising mothers with a child or children aged seven years or less. Such mothers numbered 0.71 million.

Three surveys were carried out to measure the levels of knowledge among the public prior to, during, and after the one-year campaign. These helped to gauge the degree of effectiveness of the operation in raising nutritional knowledge with respect to proteins. Some specific items may be highlighted:

1. As many as 2.7 million people in Maharashtra, or 53 per cent of the total adults in the population, were aware of the PFAI advertising campaign. This includes an important group of mothers, those with at least one child in the home younger than seven.

2. Some 20 to 30 per cent of these people were motivated to modify their diets; similar numbers talked about the campaign with their families, friends, and doctors.

3. Although the offer of a booklet entitled, "Protein is Life" was not conspicuous in the ads, 30,000 copies were mailed in response to requests emanating from a single state. This can be compared with one of 7,000 requests a year from the whole country for a booklet on dental care advertised along with a brand of toothpaste.

4. Each booklet received was read by at least seven people.

5. Statistically significant increases in protein awareness occurred. It did not occur for such other nutrition concepts as vitamins, minerals, and calories. At the end of the campaign, protein outranked these in importance in the public estimation.

6. As many as 50 to 60 per cent of the adult population had become aware of the special importance of protein for weaned children, expectant women, and nursing mothers. Correct beliefs regarding proteins have increased and, perhaps even

more important, wrong beliefs have been corrected.

7. There has been almost a doubling at all income levels in knowledge of the important concept that six months is the right age to start solid food.

8. The campaign has been least effective in the smallest towns. It has brought out the great desire for nutritional knowledge among those of lower educational level and income, as judged by the profile of those requesting the booklet. The major weakness of the campaign has been that sources of dietary protein were not adequately conveyed to the reading public.

9. The cost of the entire campaign, divided by the number of effective contacts, yields a cost per contact of only 28 paise (100 paise = 13 US cents).

One may fairly conclude that the one-year pilot campaign has brought about a significant improvement in both public awareness of proteins and the quality of this knowledge. It also shows that when imaginatively conceived and creatively used, mass media in India offer an effective means of imparting nutritional knowledge to vast numbers of people. The cost of such teaching is also extremely low.

NEWS

La Leche Program Offers Breast-feeding Information by Edwina Froehlich*

Each year, La Leche League International informs and encourages thousands of mothers to breast-feed their babies. The program is carried out through monthly meetings at which mothers share their experiences and are provided with information and encouragement. More than 2,500 such groups now meet regularly in a number of countries.

In addition to personal instruction, LLL offers information, via its own publications and other specialized literature, to parents, physicians, nurses, hospitals, and schools. Experienced League mothers are available to speak free of charge to institutions that request them.

An important aspect of the service is the Physicians' Seminars, which have been accredited by several medical organizations. These have been helpful in informing physicians of new developments.

In 1975, Paul Györgi Award contests were initiated to encourage significant research in all aspects of breast-feeding, and to interest medical students who have made outstanding contributions to breast-feeding literature.

To help the mothers who reside in developing countries, where monthly meetings often are not feasible, the League has initiated La Leche League Information Service Centers. These may be managed by any one of a variety of interested persons. At present, 19 such Centers are in operation throughout the world, each of which is designed to encourage mothers to breast-feed.

Each new Center receives a packet of printed materials, a copy of The Womanly Art of Breastfeeding, a variety of reprints and information sheets, and subscriptions to LLL News and Leaven, the bimonthly publication for LLL leaders. Each center has access to LLLI's Professional Advisory Board.

Except for the administrative and clerical workers at the international office, La Leche League is an entirely volunteer organization. A subscription to the Information Service is $12.00 for the first year and $8.00 for each succeeding year. Service Centers may order additional LLLI materials at the Leaders' discount price.

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*Executive Secretary - La Leche League International, 9616 Minneapolis Avenue, Franklin Park, Illinois 60131, USA.

International Grain Legume Information Centre established at IITA

Recent years have seen a revival of interest in the grain legumes as potentially important protein sources to supplement traditional high-starch diets in many parts of the world, especially in the tropics. A number of lesser-known varieties, including cowpeas (Vigna spp.), lima beans (Phaseolus lunatus) and yam beans (Pachyrrhyzus spp.), had been virtually neglected by agricultural researchers in the past but are now starting to receive the attention needed for realizing their potential. A growing number of research institutions are working on the grain legumes, but so far there has been insufficient coordination of efforts and inadequate provision for exchanges of information among researchers.

To counter this situation, the International Grain Legume Information Centre (IGLIC) was recently established at the International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria, with the joint sponsorship and financing by the International Development Research Centre of Canada (IDRC).

IGLIC is now proceeding to gather old and new scientific literature on grain legumes, particularly those that can be suited to the tropics. The texts are being abstracted, indexed and stored in a system from which

(News continued on page 47)

PAG DOCUMENTS

1. Documents on single cell protein issued by PAG. Contents: 1976
   a) Statement No. 4 on SCP, 1972
   b) Report of 2nd PAG ad hoc working group meeting, 1971
   c) Report of 3rd PAG ad hoc working group meeting, 1973
   d) Report of 4th PAG ad hoc working group meeting, 1973
   e) Report of 5th PAG ad hoc working group meeting on clinical evaluation and acceptable nucleic acid levels of SCP for human consumption, 1975
   f) Guideline No. 6 for Preclinical testing of novel sources of protein, 1972
   g) Guideline No. 7 for Human testing of supplementary food mixtures, 1972
   h) Guideline No. 12 on the Production of SCP for human consumption, 1972
   i) Guideline No. 15 on Nutrition and safety aspects of novel protein sources for animal feeding, 1974
2. Documents on feeding the preschool child issued by PAG ad hoc working group meetings (1969-1975). Contents: 1976
   a) Report of 2nd PAG ad hoc working group meeting, 1970
   b) Report of 3rd PAG ad hoc working group meeting, 1971
   c) Report of PAG ad hoc working group meeting on milk intolerance - nutritional implications, 1971
   d) Report of 4th PAG ad hoc working group meeting, 1972
   e) Report of 5th PAG ad hoc working group meeting, 1975
   f) PAG Statement No. 17 - Low lactase activity and milk intake, 1972
   g) PAG Statement No. 18 - Relationship of pre- and postnatal malnutrition in children to mental development, learning and behavior, 1972
   h) PAG Guideline No. 8 - Protein-rich mixtures for use as weaning foods, 1972
3. Mass communication: Report of PAG ad hoc working group meeting, New York 1975
4. Overcoming problems in infant and young child feeding practices: PAG regional seminar, Singapore 1974
5. Pediatrician/infant food industry seminar, New York 1973
6. Nutritional improvement of food legumes by breeding 1973
7. Manual on feeding infants and young children by Cameron and Hofvander 1972
   (2nd edition: 1976)

PAG DOCUMENTS*

No.	Title	Year
	Statements	
2	Recommendation on aflatoxin (S)	1969
3	Nature and magnitude of the protein problem (S)	1971
4	Single cell protein (F, S)	1970
5	Marketing and distribution of protein-rich foods (S)	1971
6	Milk substitutes (S)	1970
7	Recommendation on prevention of food losses and protein-calorie malnutrition (S)	1969
8	Plant improvement by genetic means	1970
9	Amino acid fortification of foods (F, S)	1970
10	A systems approach to the formulation and evaluation of nutrition intervention programs (S)	1970
11	Leaf protein concentrate (S)	1970
12	The world protein problem: research and development needs (S)	1972
13a	Review of the specific proposals contained in ACAST report "International Action to Avert the Impending Protein Crisis", United Nations, 1968 (S)	1971
14	Marketing of conventional foods (S)	1971
15	Popular participation and community involvement in nutrition improvement programs (S)	1971
16	The potential of fish protein concentrate for developing countries (F, S)	1971
17	Low lactase activity and milk intake (F, S)	1972
18	Relationship of pre- and postnatal malnutrition in children to mental development, learning and behavior (S)	1972
19	Maintenance and improvement of nutritional quality of protein foods (S)	1972
20	The "protein problem" (F, S)	1973
21	Specifications for solvents (S)	1972
22	Upgrading human nutrition through the improvement of food legumes (F, S)	1973
23	Promotion of special foods (infant formula and processed protein foods) for vulnerable groups (F, S)	1973
24	The Green Revolution and protein supplies	1973
25	The global maldistribution of protein: a growing trend (F)	1973
26	Food and nutrition considerations in national economic planning	1973
27	Mass communications in nutrition education (F, S)	1974
28	Issues for the World Food Conference, the PAG view (F, S)	1975
	Guidelines	
2	Preparation of food-quality groundnut flour	1970
4	Preparation of edible cottonseed protein concentrate	1970
5	Edible, heat-processed soy grits and flour (F, S)	1969
6	Preclinical testing of novel sources of protein (F)	1972
7	Human testing of supplementary food mixtures (F)	1972
8	Protein-rich mixtures for use as weaning foods (F)	1972
9	Fish protein concentrate	1971
10	Marketing of protein-rich foods in developing countries (F, S)	1971
11	Sanitary production and use of dry protein foods (F, S)	1972
12	Production of single cell protein for human consumption (F)	1972
13	Preparation of milk substitutes of vegetable origin and toned milk containing vegetable protein	1972
14	Preparation of defatted edible sesame flour	1972
15	Nutritional and safety aspects of novel protein sources for animal feeding (F, S)	1974
16	Protein methods for cereal breeders as related to human nutritional requirements (F, S)	1975

*The letters in parentheses indicate those documents that have been translated into French (F) or Spanish (S) or both (F, S).

Most PAG Statements and Guidelines have been published in the PAG Bulletin:

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NEWS

(News continued from page 42)
they can be readily retrieved. This venture is appropriate for IITA, which specializes in tropical grain legume research and already has strong information resources. The work will complement that of AGRIS, FAO's international information system for agricultural sciences and technology, which has begun to develop networks of international centers of specialized information.

Through IGLIC, IITA will publish annotated bibliographies and will provide a variety of specialized information services and guidance to agencies, projects, scientists and experts working on grain legumes in all parts of the world. A new quarterly publication, Tropical Grain Legume Bulletin, was launched in July 1975. It offers research and extension workers a means of communication for exchange of information and contains brief research reports and news as well as abstracts of current literature.

Further information may be obtained from the International Grain Legume Information Centre, International Institute of Tropical Agriculture, P.M.B. 5320, Ibadan, Nigeria.

ERRATA

PAG Bulletin, Vol. V, No. 1, p. 46 (March 1975):

NEWS:

Whey - An Important Potential Protein Source -

*Edited summary from a note bearing the same title, which was published in the Monthly Bulletin of Agricultural Economics and Statistics, Vol. 22, No. 9, April 1974

should read

Vol. 23, No. 4, April 1974

in English, French and Spanish issues.

PAG Bulletin, Vol. V, No. 4 (December 1975):

Back cover, first column, line 3:
Change word "was" to "will be".

PROTEIN-CALORIE ADVISORY GROUP

The Protein-Calorie Advisory Group of the United Nations System (PAG) is an interdisciplinary committee of internationally-recognized experts who advise the United Nations and its agencies on technical, economic, educational, social and other related aspects of global malnutrition problems and the broad programs and new areas of activity needed for combating them. Since its inception in 1955, the PAG has emphasized protein-calorie malnutrition as a primary and continuing threat to the health and survival of infants and young children in the developing countries and has played an active role in promoting the development of novel and locally-available protein resources for the developing world. The PAG also reacts to socioeconomic considerations, trends in world food supply and consumption and the need for governmental initiatives and priorities in dealing with these problems.

The PAG is sponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Bank for Reconstruction and Development (IBRD), and the United Nations.

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The purpose of the PAG Bulletin is to promote the exchange of information on the world malnutrition problem among all those who are motivated to work towards its solution. Published quarterly in English, French, and Spanish editions, it is sent gratis to individuals, institutions, and commercial organizations with an active interest in scientific, technological, economic or social aspects of protein-calorie malnutrition on a worldwide basis.

The PAG Bulletin can succeed in its mission only insofar as it can comprehensively and objectively communicate with its readership. Readers are invited to comment in writing on what they read in the Bulletin. In addition, the PAG Secretariat welcomes suggestions for broadening and deepening the scope of the Bulletin, thereby increasing its usefulness.

Mailing list
Every effort will be made to honor requests to be added to the PAG Bulletin mailing list. In the interests of economy, however, copies should be shared by colleagues or borrowed from libraries wherever possible. Address requests to:

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VOLUME VI, NUMBER 2, JUNE 1976

PAG BULLETIN

Protein-Calorie Advisory Group
of the United Nations System

PAG Bulletin

Volume VI, Number 2, June 1976

COVER:

Ethiopian children enjoying their food at relief center in Wollo Province. (UNICEF photo by John Balcomb.)

IN THIS ISSUE....

PAG Bulletin. Vol. VI, No. 2. June 1976. *PAG Bulletin* is published quarterly in English, French and Spanish editions by the Protein-Calorie Advisory Group of the United Nations System, Room 606, 866 United Nations Plaza, New York, N.Y. 10017, U.S.A. Controlled circulation postage paid at New York, N.Y., U.S.A.

PAG BULLETIN

VOLUME VI, NUMBER 2
JUNE 1976

PAG ad hoc WORKING GROUP MEETING ON SINGLE CELL PROTEINS

The ad hoc Working Group meeting, the sixth in the series, was held in Chateau Ste. Anne, rue du Vieux Moulin 1160, Brussels, Belgium, on 31 March and 1 April 1976. The meeting was opened by the Chairman, Professor N. S. Scrimshaw, who welcomed the Working Group members and other participants, listed below.

The information presented and discussed at the Symposium on Suitability of Hydrocarbon Grown SCP for Animal Feeding held on the preceding two days was reviewed and the following specific issues were considered at length. A summary of the proceedings of the Symposium presented by Professor N. S. Scrimshaw appears elsewhere in this issue.

Fatty acids with uneven numbered C-atoms in SCP

It is known that some SCP products contain fatty acids of uneven numbered carbon chain length (UCFA). These are also present in many other materials in common use including marine oils and mammalian milk. As might be expected, these UCFA are found in the fat of various organs and in particular, in the adipose tissue of animals fed materials which contain high levels of UCFA.

The intermediary metabolism of these UCFA has been known for several decades and, in general, is parallel with that of the more common even numbered fatty acids. The β-oxidation system of the mitochondria is responsible for transformation of the odd and even numbered saturated and unsaturated fatty acids into metabolic end products. The exchange of lipids between cellular membrane systems takes place rapidly. Normal brain cerebrosides and sulfatides contain large quantities of long chain odd-numbered normal α-hydroxy monoenoic fatty acids. Long term studies using SCP containing traces of odd carbon fats have shown no evidence of toxic responde attributable to anamolous fat metabolism in several organs and tissues studied.

More recently, experimental studies have shown that the presence of UCFA resulting from feeding certain SCP products does not exceed 10 per cent of the total fatty acids in any tissue examined. This percentage is well below the capacity of the body to metabolize and utilize UCFA. In addition, functional studies even on stressful conditions carried out on all the major organs and tissues of experimental animals have produced no evidence to suggest that any difference exists in this respect between those containing UCFA and those containing fatty acids of even-numbered carbon chain length.

Residual n-paraffin

Chemically, paraffins represent several entities and there is evidence that they are present in many foods commonly consumed by human beings. Body fat composition is responsive to dietary fat - a fact known to all animal feeders. It is not appropriate to speak of changes in body fatty acid composition with diet as residues since all fatty acid compounds are transformed into metabolic products according to the nature of fat consumed and according to physiological demand.

Microorganisms and plants contain hydrocarbons of various types resulting from biosynthesis. In the case of SCP derived from alkane substrates, the level of paraffins which they contain is generally in the range of 1 part in 1,000 (0.1 per cent) and 1 part of 200 (0.5 per cent) of the total weight and never above 0.5 per cent. It is not surprising, therefore, to find a proportion of these paraffins in the fat of some species of animals which have been fed diets containing this type of SCP.

The paraffins in the fat of animals fed yeast grown on normal paraffins are within the limits of normal variation. In fact, much higher levels have been found in the fat of cattle fed entirely on grass. Animals fed SCP for prolonged periods at very high levels showed in the fat depots C-14 to C-40 hydrocarbons, and over 76 per cent of these were between C-14 and C-24. Continuous feeding of n-paraffin did not give rise to a progressive increase in the accumulation of these compounds,but after an initial period of increase, n-paraffins participated in the biological oxidation process. The enzymes oxidizing paraffin hydrocarbons were found to increase as an adaptive mechanism under these circumstances.

Although, for many years, linear alkanes were regarded as being biologically inert, it is now known that if absorbed they can be oxidised to the corresponding fatty acids by enzyme systems existing in the animal body. This is mor readily achieved with the liquid than the solid alkanes, that is to say with alkanes of the carbon chain length residual in the SCP grown on hydrocarbons.

Such evidence as is now available indicates that the level and nature of the residual paraffins in SCP present no hazard to the health of animals fed on these materials, provided a feedstock of adequate purity is used in the cultivation of the biomass. Neither is there any evidence of hazard to the health of consumers of products from these animals resulting from the presence of such paraffins as may have been transferred to these products from the SCP. Indeed, in some cases, this is below the level of paraffins found in the fat of animals which have been fed on diets containing no SCP.

Recent studies have demonstrated that the paraffins extracted from alkane-grown SCP have no deleterious effect on the viability and respiratory function of subcellular organelles nor do they have any mutagenic effect upon the strains of S. typhimurium which have been developed as test organisms for this purpose. These confirm the long term feeding and multiple generation studies on the intact animal which have already been reported to the PAG.

The use of n-paraffins in a number of staple food preparations is allowed in most countries. The level of food grade mineral oil which is permitted in the processing of foods such as bread, rice, meat, leaves residues many times higher than has been found in the fat of animals fed on SCP grown on alkanes and no harmful effects have been reported as resulting from this widespread and long standing practice.

The PAG recommends that analytical methods for determining the level and composition of the residual paraffins in alkane-group SCP should be standardized so as to facilitate a valid comparison of the results reported by different workers. It would welcome new or further information on the presence and nature of n-paraffins in natural products which would, of course, include the tissues and products of animals fed SCP grown on alkanes.

Strain stability

Concern has been expressed on the possibility that a microbial strain used for the production of SCP could present mutational changes resulting in the acquisition of toxic or other unfavorable properties. Actually, the chances for such transformation are minimized by the fact that in continuous cultivation for biomass production conditions tend to eliminate any mutation of this sort which has a lower growth rate and growth efficiency. Thus strain stability is favored.

Experience accumulated in long-term continuous cultivation of SCP either at the pilot plant level or at the scale of full size production unit have confirmed those expectations and demonstrated the remarkable stability of the strain, provided

that the conditions of culture are maintained constant.

In addition, a variety of methods exist which permit control of the stability of a microbial strain by biochemical and immunological procedures, including DNA hybridization and enzymological testing. Manufacturers and responsible government authorities must control the identity of the SCP products with those for which permission for commercialization has been granted.

In order to verify that a commercially distributed SCP conforms in all respects to the product which has been initially tested and authorized, the manufacturers should be made to deposit the concerned strain of the microorganism in an official culture collection prior to permission for manufacture. Necessary consideration should be given to protect the confidentiality of the strain deposited. This would facilitate checking the identity whenever necessary by the regulatory authorities as well as by the manufacturers themselves. The precautions to be taken for control of identity are a) testing and manufacture should be done on the same strain of organism, and b) regular checking for any changes in long range production.

The meeting was informed that the World Federation of Culture Collection is the representative body and there are fifty to sixty national culture collection centers around the world. The group was informed that in Japan attempts are being made to develop laboratory identification using sensitive chemical methods.

It was proposed that the International Association of Microbiological Societies (IAMS) should be requested to prepare a statement on the present status of available scientific methods to check the stability of microbial organisms used for industrial SCP production and advise on functional details regarding type culture collection as a repository for reference purposes.

Reviewing the future role of PAG relating to SCP research and development, the Working Group stated that the technology for production of SCP is well established at present. SCP products are sold for animal feeding in many West European countries without restriction except the usual quality controls. Apart from specific problems of a technological nature, which are identified or brought to its attention, the PAG Working Group should examine in the future the following related issues.

1. The growing pressure of environmental problems associated with wastes from agriculture or food and animal industries underlines the urgency of reclaiming such biological waste materials into useful resources as animal feed ingredients, including SCP. Advanced microbiological technologies are available for this purpose and are already in use in many countries. The Working Group would like to draw the attention of the PAG to the need for monitoring developments in this field so that the nutritional and safety aspects of the technologies and the resulting products can be evaluated. The PAG efforts in this area should be undertaken with the collaboration of other international groups. Since these technologies are of great interest to developing countries, the Working Group noted with interest that FAO/UNEP is holding a meeting on waste utilization for feed, food, fertilizer and fuel, in the near future.

2. Besides the major industrial processes for large scale production of SCP and the utilization for animal feed of agricultural and animal wastes through appropriate microbiological technology, protein enrichment by fermentation of starchy materials (in particular the dry fermentation of cassava, banana, potatoes, etc.) can be developed. They provide new possibilities for improving significantly the protein food supplies of developing countries. Traditional products of this type are in large scale use for direct human consumption in some developing countries. For example, Tempe, a fermented soy product, and Ontjon, a fermented peanut cake, have been popular foods for centuries in S. E. Asia. The Working Group considered these developments significant and proposed that they be reviewed at a future meeting.

3. Progress achieved in research, development and industrial production of SCP for animal feeding is attracting considerable interest in

both developed and developing countries largely because of the rising prices of conventional animal feeds. Although PAG has looked into the nutritional and safety aspects of SCP for animal feeds and has made useful and practical recommendations, the economic aspects of these new developments have not been reviewed recently. Precise information in this area would be of significance to various countries for agricultural and food planning. Future PAG Working Group meetings should undertake a review and appraisal of the economic aspects of SCP production under different situations and develop suitable models for the economic feasibility of different processes for SCP production. Meanwhile, it is essential that every country, before embarking on this venture, should examine carefully the economic feasibility of SCP production from hydrocarbons.

Discussing the problems of consumer acceptance of SCP, the Working Group stated that the PAG concern for research and development in SCP covers the needs for the whole world, the use of all possible substrates and the use of the product both for animal and human feeding. While the Working Group cannot involve itself in making statements on particular situations prevalent in specific countries, it can conduct a scientific review and make appropriate scientific statements. One way the PAG can be helpful in this regard is to publicize all PAG documents issues on this subject. Interested organizations and groups in different countries can help in translating these documents into their respective languages for distribution to government authorities, industry personnel and others to whom these developments are of interest. National scientists engaged in research and development in this area may write popular articles providing a balanced and not exhuberant view of the subject to educate the public on these new scientific developments.

The PAG should explore the possibility of referring to an outside agency or group (Codex, IUPAC, etc.) the problem of SCP nomenclature. The nomenclature should be such that it is useful for food regulation and food laws. Similar problems in the vitamin field have been successfully solved. If results have to be obtained with SCP in the next ten or twenty years, work on this should be initiated right now.

The Working Group noted the discussion held on this subject at the 1973 meeting, the relevant portion of which is reproduced below:

"The term 'single cell protein' (SCP) was coined in 1966 as a title for the first international conference on this subject. It was chosen as a new and neutral term to avoid the unfavorable food connotations of such names as microbial protein, bacterial protein and petroprotein. It refers, however, not to protein as such but a food supplying energy and other nutrients in addition to protein.

"As a generic term, SCP has come into rather wide use to describe microcellular proteins derived from bacteria, yeasts, molds and algae, although it is not accurately descriptive of all of these materials since some are not monocellular. Moreover, in addition to proteins, they contain carbohydrates, lipids, nucleic acid, vitamins and minerals.

"The term single cell protein has served a useful purpose over the past decade when these materials were being developed for use as feed or food constituents by growing various organisms on a number of different substrates. Because of differences in protein and amino acid composition, digestibility, nutritive value and toxicological properties, it is important to distinguish among the individual products in this class. Regulatory agencies will unquestionably require more specific terminology for labeling purposes or for setting standards of identity.

"It is suggested that a term or terms be devised which will serve as the basis for a system of nomenclature to indicate both the general type of microorganism (yeasts, bacteria, microfungi, unicellular algae, filamentous algae, etc.) and the class of substrate upon which it is grown. The terms will not be substitutes for the trade names individual manufacturers are proposing, but should help the public to recognize that single cell proteins may be as different as fruits or

vegetables, or meat or fish. It is believed that such a system of nomenclature would be informative not only to technologists and regulatory agencies, and ultimately to consumers, but would have special advantages over such terms as 'petroprotein' or 'bacterial protein.' If meaningful and non-pejorative nomencalture is not adopted, the acceptability of 'single cell protein' and hence the practical success of enterprises to produce and market these useful nutrients may be jeopardized by consumers' misunderstanding."

There was a general discussion on allergic and other toxic reactions in human beings fed SCP and the following points were made.

While untreated Candida utilis has not caused any allergic reaction, several of the types of yeasts and a bacterium fed to human beings led to severe allergic response. In a recent unpublished study, C. utilis processed in one way to remove RNA gave rise to cutaneous allergy and processed in another way did not cause any reaction. This report of occurrence of cutaneous allergy instead of alimentary allergy is considered noteworthy, and it is hoped these results can be published soon.

The manifestations of allergy in animals are not the same as those observed in humans and may be nonspecific (such as loss of weight) and difficult to correlate with the allergen. Since substances other than protein can also cause allergy, tests for identifying the allergen should be extensive and include all lipo-poly-saccharides and other types of potential allergens. The Working Group had no answer to the questions whether the meat from animals fed SCP would cause allergy in the human consuming the meat and whether such meat is more allergenic than that derived from non-SCP fed animals.

Extensive clinical testing was considered essential prior to human feeding of SCP material and following thorough preclinical testing. Otherwise, regulatory authorities should not pass the substance for human use. It is essential to have standardized testing procedures for identifying all reactions before the products are allowed to be marketed. Even if and when such products have passed the allergy tests and allowed to be marketed, information should be monitored on a regular basis. It was noted that the people working in factories producing SCP are regularly monitored for allergy to dust in the atmosphere (occupational hazard). It was recommended that PAG establish a special ad hoc Working Group to review the allergy and other toxic problems relevant to human feeding.

In this connection, the invitation extended to PAG by Dr. S. Garattini, Director of the Istituto di Ricerche Farmacologiche "Mario Negri" for holding a working group meeting in Milan, Italy, was received with thanks. The meeting will review and summarize all previous and current research work on clinical and metabolic data on the use of SCP.

List of Participants

Members

Dr. B. L. Oser, Bernard L. Oser Associates, Inc., Forest Hills, New York, USA.
Professor A. A. Pokrovsky, Academy of Medical Sciences, Moscow, USSR.
Professor N. S. Scrimshaw, Massachusetts Institute of Technology, Cambridge, Massachusetts, USA.
Professeur J. C. Senez, Centre National de la Recherche Scientifique, Marseille, France.
Professor A. G. Ward, The University of Leeds, Leeds, England.
Dr. K. Yamada, Sapporo Breweries Ltd., Tokyo, Japan.

Consultants

Professor S. Garattini, Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy.
Dr. K. Katoh, Ministry of Agriculture and Forestry, Tokyo, Japan.

FAO

Dr. G. D. Kapsiotis, Food Policy and Nutrition Division, Rome.

WHO

Dr. E. M. DeMaeyer, Nutrition Unit, Geneva.

Observers

Dr. A.P. de Groot, Central Institute for Nutrition and Food Research, Zeist, The Netherlands.
Dr. Van Weerdan, ILOB, Wageningen, The Netherlands.

Invited Guests (attended the meeting on 31 March only)

Dr. Max Milner, U.S. Congress, Washington, D.C., USA.
Dr. C. A. Shacklady, BP Proteins Limited, London, England.
Dr. Samuel Shibko, U.S. Food and Drug Administration, Washington, D.C., USA.
Dr. D. A. Stringer, Imperial Chemical Industries Limited, Berkshire, England.

PAG SYMPOSIUM ON HYDROCARBON - GROWN SCP PRODUCTS FOR ANIMAL FEEDING: A SUMMARY*

The development and use of single cell protein (SCP) has been proceeding for many decades. But, apart from wartime situations, it is only in the last decade that it has achieved major new impetus, with the use of hydrocarbon substrates coming into prominence.

It has been pointed out that Western Europe meets only 20 per cent of its feed protein needs, and according to Professor J. C. Senez of France's Centre National de la Recherche Scientifique, it is importing 8.2 million tons per year of protein concentrates, mainly soybean meal from the USA, costing $4,000 million. Any import substitution that is economically viable would be desirable. In addition, the USSR has major need for protein concentrates for animal feeding.

It was clear from the presentations at the Symposium that there are three products in Western Europe - Toprina, Pruteen and Liquipron - which have undergone very extensive testing in laboratory and farm animals and for which large scale production plants exist. For Toprina and Liquipron, which are both yeasts grown on n-paraffins, by British Petroleum and Liquichimica respectively, plants with capacity of 100,000 tons per year have been constructed in Italy and are awaiting government approval. In addition, Toprina production using a n-paraffin substrate (contained in gas oil) has proceeded in an 18,000 tons per year plant at Lavera, France.

Production continues in a 4,000 tons per year plant in Grangemouth, Scotland, using an n-alkane substrate. In four years of commercial sales for calf, poultry and swine feeding, the material has been well accepted and no problems have been encountered. For Pruteen, a bacterial product grown by Imperial Chemical Industries on methanol, future plans depend on economic trends. In addition, Shell is investigating the use of methane as a substrate employing multiple organisms.

In the USSR, intensive research has led to the identification of efficient organisms, and extensive analytical and biological evidence of their safety for animal feeding has been obtained. The Symposium was informed that a plant capable of producing at least 100,000 tons per year was completed in 1973, and additional plants of this

*The Symposium was held on 29 and 30 March 1976 in Brussels, Belgium, to review technical developments and present status including regulatory aspects pertinent to SCP production from hydrocarbon substrates and for use in animal feeding. This Summary was presented by Professor N. S. Scrimshaw, Chairman of the PAG ad hoc Working Group Meeting held on 31 March and 1 April and Co-Chairman of the Symposium, at the closing session of the Seminar.

size were completed in 1974 and 1975. Several more large plants are planned.

As to toxicological testing of these materials, the Protein-Calorie Advisory Group of the United Nations System (PAG), developed guidelines for the nutritional and safety evaluation of new protein sources for human feeding, issued in 1972, and a separate guideline for the evaluation of SCP for animal feeding was issued in 1975. A complementary set of recommendations relating to SCP for animal feeding has been prepared by International Union of Pure and Applied Chemistry (IUPAC).

As summarized by Dr. B. L. Oser of Bernard L. Oser Associates Inc., a Consultant to the U.N., these guidelines call for extensive analyses to ensure appropriately low levels or absence of heavy metals and other undesirable substances. Also required are tests of the microbiological stability of the organism; freedom from microbiological contamination; adequate nutritional value and acceptable freedom from toxicity - as determined by short and long-term tests using experimental and target animals - plus multi-generation studies; and special tests for teratological or mutagenic properties; and practical feeding experiments.

Dr. A.P. de Groot of Holland's Central Institute for Nutrition and Food Research (ILOB) and Dr. D. Stringer of Imperial Chemical Industries presented an impressive array of biological results from rats, mice, quail, pigs, chickens, calves, monkeys and other animals attesting to the safety of the SCP materials evaluated. Dr. de Groot also spoke of SCP feeding studies on 19 generations of rat, with reproductive studies within each generation, and on 28 generations of quail.

The PAG Guidelines have been followed with extraordinary care, diligence and expense. No food or feed product on the market today has been so thoroughly tested for safety as those discussed at the meeting.

Since the extensive studies of normal growth, reproduction, life span, performance trials and of substrate purity have not been enough to satisfy all doubters, attention was focused on four specific issues. The first of these was the matter of strain stability. Could there be mutations of the organisms involved which might pose a health hazard? It was noted that the Guidelines call for detailed control procedures, both morphological, chemical and biochemical, to detect such change and thus ensure safety. Moreover, the required control procedures of good manufacturing practice are such that, in the remote chance that such a change occurs, the material could be stopped from reaching the market. It was noted that in the years of experience with the organisms described at the meeting, no such mutation had been observed. Furthermore, from a production point of view, the most likely consequence of a mutation would be an alteration in productivity, and there is as much concern for maintaining strain stability for high productivity as for safety - the two go together. Periodic biological tests for toxicity are also done.

The second issue relates to the occurrence of fatty acids with an uneven number of carbon atoms in the chain. Elegant studies by Dr. S. Garattini of Italy's Istituto di Ricerche Farmacologiche "Mario Negri" have shown that these are transformed into metabolic products (as are the more common even numbered fatty acids); are available for the stress response; and are unassociated with any changes in brain, heart, liver, blood platelets and a series of other tissues. Moreover, there are many earlier studies that have shown these uneven-numbered carbon-chain fatty acids to be constituents in many other foods, and are metabolised by way of physiologically normal pathways. These acids are normal constituents of many other foods.

Dr. C. A. Shacklady, BP Proteins Limited, provided some interesting supplementary evidence when he reported that such fatty acids had no detectable effect on mouse peripheral macrophages, on rat liver mitochondria respiration, and no mutagenic activity with five different strains of test organism.

Third is the question of residual n-paraffin, that is saturated linear chains of carbon

atoms, particularly in the range of C 18 to C 33. Body fat composition is responsive to dietary fat - a fact known to all animal feeders. It is not appropriate to speak of these changes in fatty-acid composition with diet as residues, since they are transformed into metabolic products as are the more common even numbered fatty acids according to the nature of the fat in the diet and according to physiological demand. Microorganisms and plants contain hydrocarbons of various types resulting from biosynthesis. In the case of SCP derived from alkane substrates, the level of paraffins which they contain is generally in the range of 1 part in 1,000 (0.1 per cent) to 1 part in 200 (0.5 per cent) of the total weight and never above 0.5 per cent. It is not surprising, therefore, to find a proportion of these paraffins in the fat of some species of animals which have been fed diets containing this type of SCP. The paraffins in the fat of animals fed yeast grown on n-paraffin are within the limits of normal variation. In fact, much higher levels have been found in the fat of cattle fed entirely on grass.

Although, for many years, linear alkanes were regarded as being biologically inert, it is now known that if absorbed they can be oxidised to the corresponding fatty acids by enzyme systems in the animal body. This is more readily achieved with the liquid than the solid alkanes, that is to say with alkanes of the carbon chain length residual in the SCP grown on hydrocarbons.

Such evidence as is now available indicates that the level and nature of the residual paraffins in SCP present no hazard to the health of animals fed on these materials, provided a feedstock of adequate purity is used in the cultivation of the biomass. Neither is there any evidence of hazard to the health of consumers of products from these animals resulting from the presence of any paraffins that may have been transferred to these products from the SCP. Indeed, in some cases, this is below the level of paraffins found in the fat of animals which have been fed on diets containing no SCP.

Recent studies have demonstrated that the paraffins extracted from alkane-grown SCP have no deleterious effect on the viability and respiratory function of subcellular organelles nor do they have any mutagenic effect upon the strains of S. typhimurium which have been developed as test organisms for this purpose. These confirm the long term feeding and multiple generation studies on the intact animal, which have already been reported to the PAG.

The use of n-paraffins in a number of staple food preparations is allowed in most countries. The level of food grade mineral oil which is permitted in the processing of foods such as bread, rice and meat leaves residues many times higher than has been found in the fat of animals fed on SCP grown on alkanes and no harmful effects have been reported as resulting from this widespread and long standing practice.

The fourth special topic is a false issue. Since benzpyrene and other aromatic hydrocarbons can be induced in crude petroleum by high temperature cracking, the use of a petroleum-derived substrate brings with it the spectre of possible carcinogenesis. However, as Dr. Oser pointed out, the feedstocks used for SCP production are free of such substances, as judged by the most sensitive gas-chromatographic analysis available; that is, they have less than one part per thousand million and perhaps none.

The question then arises: could the organism concentrate traces of such materials not detected in the SCP products themselves? and could these undetectable levels be carcinogenic? The answer is that, in experimental studies with direct feeding of these aromatic hydrocarbons, it takes far more than one part per thousand million to begin to have detectable carcinogenic effects. Moreover, no carcinogenic effects turn up in multi-generation studies and in lifetime studies, where these materials have been fed at levels well above those which would be used in normal commercial feeding. These studies have been carried out in a number of different countries.

Could these materials somehow get into the fat of animals fed SCP? The answer is no again,

according to the most sensitive chemical analyses available. However, traces of these aromatic hydrocarbons do get into our environment in many ways, including traces in in some feeds and food supplies and turn up in trace amounts from other sources. There is no indication that SCP prepared in the ways discussed would add to that hazard.

It can thus be concluded from the Symposium discussions that there is no current scientific ground for objecting to SCP as animal feed on the basis of any of these four special issues. Moreover, there is no evidence that more work is required to settle any of these issues for the materials described, although further research will be required as each new SCP product is proposed for animal feeding.

An additional special point strongly emphasized in the PAG Guidelines was mentioned by Professor L. Ray of Nestlé Alimentana. That is, that no set of animal tests can be a substitute for cautious and systematic tolerance studies in human subjects when any new SCP material is intended for direct feeding, as is true for any new protein source. This is because there can be allergic reactions in human subjects which cannot be detected - at least up to this point - in experimental animals. There must also be taste and functional characteristics not necessary in a material for animal feeds.

The next point deals with government regulations. Governments must have, and most do have, reasonable procedures for processing an application, for evaluating the scientific data or having it evaluated by competent advisory groups, and for prompt and clear decisions, either affirmative or indicating such additional data as may be required. The UK has a slight variant of this in which more responsibility is placed on the producer. In any event there is a requirement for strict internal laboratory controls on the part of the industrial concern and also for external controls by the appropriate government agency. The PAG and IUPAC Guidelines are intended to facilitate this process for both government and industry.

In evaluating scientific data the PAG looks critically at all data, regardless of source. Excellent scientific contributions to these subjects have come from universities, from research institutes, from government laboratories and industrial research laboratories. The criterion is the quality of the scientific work, not where it is done. On the basis of the data which have been obtained, Toprina can now be sold in the UK, France, West Germany, Holland, Belgium, Denmark, Luxembourg, Switzerland, Spain and Portugal as animal feed, without restriction other than normal quality controls. In the USSR, the SCP produced on n-paraffins (designated BVK), is used at a level of several hundred thousand tons per year. The problem comes when factors other than scientific considerations and the national and public health interest, intervene to mislead or arouse public opinion or to delay or block decisions - this the PAG Working Group can only deplore.

No blanket statement should be made about SCP because it is a generic term covering many different possible organisms, substrates and processes. Only certain organisms, substrates and processes will prove suitable for animal feeding. It can be stated, however, that man's experience with similar products - brewer's and baker's yeast - over centuries, and with Candida utilis grown on various carbohydrate substrates - establishes the principle of safe use of selected SCP products.

Now it has been demonstrated that selected yeasts and bacteria grown on purified hydrocarbon fractions such as normal alkanes and methanol can also be safe and nutritious for animal feeding and the resulting animal product used in human diet. It can be concluded that as far as these particular materials are concerned, there are no nutritional or toxicological barriers to their commercial development. Their future should depend entirely on economic viability and the public interest.

CURRENT STATUS OF ISSUES RELEVANT TO SINGLE CELL PROTEINS IN JAPAN

Koichi Yamada*

1. The Food Sanitation Investigation Council, an advisory organ to the Health Minister in the area of Food Safety, declared on December 15, 1972 the safety of two SCP products of the Kanegafuchi Chemical Co. and the Dainippon Ink and Chemicals Co. respectively. Under the authorization of the Ministry of Health and Welfare, the safety declaration based on the investigations for more than two years is still valid.

2. On the request of the Health Ministry, the Ministry of Agriculture and Forestry undertook in 1974 a special research project entitled "Monitoring Methods for Yeast Destined for Animal Feeding." Under this program the nutritional and safety evaluation of SCP yeast based on alcohols as substrate is being conducted and the methods are being developed for establishing the identity of SCP microorganisms.

3. The international emergence of various SCP technologies, including those based on wastes has, during the years 1973 and 1974, deeply sensitized the nation's central organ of agricultural and food research to the potentials of SCP as a protein resource for Japan. In 1975, the Agricultural Research Council of the Ministry of Agriculture and Forestry initiated a five year integrated project called "Development of Feed Resources from Agricultural and Fishery Wastes." (See list below.) In this big project, the Agricultural Ministry is attempting to establish a system for monitoring all kinds of SCP based on unconventional substrates by means of chemical, biological and microbiological testing, in parallel with the development of production technologies for SCP from a variety of wastes, such as citrus processing residue, rice straw and husk, and fish processing waste liquor. The project on yeast (item 2 above), started in 1974, was incorporated into this five year program.

4. An amendment to the Law of Commercial Feedstuffs Quality Control was passed by the National Parliament Session in July 1975. The previous legislation was based on the registration of feeds and feed additives for commercial trade. The amendment completely altered the control systems, by the enforcement of regular inspection of quality specifications according to the guidelines and standards set by government authority. This amendment provides the legal basis for approval of novel feed materials, including SCP. It will take sometime to provide all necessary guidelines specific to SCP, since the present efforts are directed primarily to feed additives including antibiotics. The responsibility for implementation rests with the Agricultural Resources Council and its Feed Committee.

5. The tentative ban on technology transfer and commercial sample export of hydrocarbon-based SCP announced by the Japanese Government is still in effect without any legislative authorization. This administrative action is one of the major inhibitory factors in SCP research and development.

6. Despite best efforts, it was not possible to invite PAG to hold the ad hoc Working Group Meeting on SCP in Japan. However, it was possible to convene an International Seminar on SCP, the Needs, Assessment and Regulations, in Tokyo on August 11, 1975, in conjunction with the Xth International Congress of Nutrition held in Kyoto. Japanese scientists acknowledge with thanks the cooperation and participation of the several members of the PAG ad hoc Working Group as well as other scientists. The credit for the success of the seminar goes to the International Development Center of Japan, and its special advisor, Dr. Saburo Ohkita, for having been able to assemble such a distinguished panel of experts

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*Professor Yamada is President, Japanese Association of Industrial Fermentation; Professor Emeritus of Industrial Microbiology, Tokyo University; and Member, Agricultural Research Council, Ministry of Agriculture and Forestry.

for the seminar.

Development and Utilization of Novel Protein Resources by Microbiological Technology - Research Programs

I. Assessment of single cell proteins

A. Identification of SCP microorganisms (chemoidentification)

1) NMR spectrum of cell wall polysaccarides
2) Serological methods
3) DNA homology
4) DNA guanine-cytosine content
5) Ubiquinone type
6) Lipid profile
7) Amino acid profile
8) Chemoidentification and morphological/ physiological examinations

B. Detection of SCP in commercial products

C. Safety evaluation of SCP

1) Pathogenicity of microorganisms
2) Multi-generation reproduction studies (hog, laying hen, fish)
3) Histopathological studies of animals and fish

D. Nutritional evaluation of SCP

1) Biological (Feeding Trials)
2) Chemical: a) Lipid Analysis, b) General Analysis

E. Monitoring of toxicity

1) Biological monitoring methods: a) target species - experimental animals, chick embryo and brine shrimp, b) data processing
2) Chemical assay of microbiological toxins
3) Carcinogenic substances
4) Heavy metals

II. Development and utilization of single cell proteins

A. Development of single cell proteins

1) SCP from citrus juice waste
2) SCP from wood waste and rice husk
3) SCP from fish processing waste
4) Algal SCP based on clean carbon dioxide exhaust
5) Koji-mold SCP based on soybean processing waste liquor and acetic acid
6) Bacterial SCP based on methanol

B. Utilization of single cell proteins

1) Feed component analysis and nutritional value

   a) Chemical Scores
   b) Hog Studies
   c) Laying Hen Studies
   d) Fish Studies

2) Biologically active factors in SCP

   a) Antinutrients
   b) Factors enhancing productivity of animals and poultry
   c) Antistress factors

3) Feed application technologies

   a) Hog feeding
   b) Pellet forming
   c) Poultry feeding
   d) Organoleptic studies of products

4) Storage of SCP feeds

   a) Storage conditions
   b) Fat deterioration
   c) Formation of secondary deleterious principles

The Working Group noted the developments and asked Professor Yamada to report to the PAG Secretariat, at periodic intervals, the progress of work and the results of the studies undertaken in each year on the research program listed above.

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ANTHROPOMETRY IN NUTRITIONAL SURVEILLANCE: AN OVERVIEW*

The nutritional status of individuals and populations is of wide socioeconomic and public health importance. In development planning it is today increasingly realized that the improvement of the nutritional status of the people should be a principal goal. In public health the assessment of nutritional status lies at the basis of all efforts to control nutritionally determined or co-determined disease.

In general, the objectives for which the nutritional status of populations and individuals need to be assessed can be summarized as:

1. diagnosis of the nature, extent and causes of nutritional problems;
2. establishment of a baseline for a later evaluation of control measures, curative or preventive;
3. the screening of populations for individual health care;
4. the recognition (identification) of changes in nutritional status as trends or movements around trends;
5. the planning and monitoring of programs and policies for food and nutrition; and
6. the establishment of an early warning system for a seriously deteriorating situation or a developing emergency.

Quite obviously the objectives will, to a certain extent, influence the choice of the methods of assessment. Thus, the first two objectives given above would be adequately served by a single survey which might be repeated after a considerable interval, whereas the latter four objectives would call for continuous or periodic assessments in the nature of surveillance and, accordingly, would call for simplified methods. The objectives would further determine the kind of sampling and the need for absolute criteria to distinguish between the normal and pathological or for relative indices of change.

However, the objectives will not be the sole determinants of the methodology employed. The type and prevalence of the major nutritional conditions, the urgency with which the results will be needed, the age groups affected, the availability of trained personnel, supporting facilities and financial resources, etc., will all influence what way which information will best be collected, as, last but not least, will the feasibility of eventual measures to improve the situation.

Today, relatively simple anthropometry is widely used for the assessment of protein-energy malnutrition in children by measuring growth, body mass and body composition -- often in relation to age. Strictly speaking, anthropometry does not specifically assess protein-calorie malnutrition. Growth and body composition are additionally influenced by genetic and disease factors that are not measurable at present. Nonetheless, selected anthropometric indicators can be proposed for gauging nutritional trends in populations. The problems start when deciding what measurements to take on which age groups and how to interpret the data.

Weight at birth

Relationships between socioeconomic status, maternal nutrition and birth weight are well documented (3,4), and there is recent evidence that supplementary food during pregnancy can increase the birth weight (5,6). An association between the weight of children at birth and their mothers' standard of living has been shown to exist not only when developed and developing countries are compared but also within the countries. In countries where malnutrition is common, a large proportion of children are

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*Extracted from a background paper, "Anthropometry in nutritional surveillance: a review based on results of the WHO collaborative study on nutritional anthropometry", written by the Nutrition Unit, WHO, Geneva, for the Joint FAO/WHO/UNICEF Committee on the Methodology of Nutritional Surveillance, which met in Geneva, 1-10 October 1975. WHO document NUT/EC/75.6 (English only).

born with a weight under 2.5 kg, as for instance in Guatemala, where in certain population groups 13-40 per cent of newborns weigh less than 2.5 kg (6, 7). A lowering of birth weight has been observed in acute famine lasting less than the total period of pregnancy (8). On the other hand, relatively small dietary improvements, of the order of 100 kcal per day, appear to raise the birth weight (6). Birth weight is influenced by a number of other factors such as socioeconomic status, number of previous children born, stature of parents, and toxic and infectious episodes during pregnancy. The weight of the newborn, apart from indicating an increased risk of fetal and postnatal death, could be used as an index of change in the nutritional status of mothers and of the community, even if the known non-nutritional influences make its value as an absolute indicator of nutritional status doubtful. The percentage of newborn children with a birth weight below a certain limit has been suggested as an index (9), and various limits have been employed: 2.0 kg (1); 2.5 kg (9); and 3.0 kg (2). In deciding on the limit to be employed the objectives of collection of the data will have to be considered.

Infants and preschool children

Most clinical malnutrition is observed in older infants and in preschool children, and accordingly this group has attracted more of the attention of nutritional anthropometry than have other age groups. This is particularly true for developing countries. However, interest in developed countries is increasing, partly because of the increasing frequency of obesity in childhood in affluent populations, partly because of concern over pockets of moderate malnutrition in disadvantaged groups.

A number of simple or composite anthropometric indices have been introduced by various workers. The more commonly known ones that will be discussed here are based on arm circumference, head circumference, weight, height, and age.

Arm circumference. Between one and four years of age arm circumference shows little increase and is therefore relatively age-independent. Little is known about the variability of this measurement. Some data from a WHO collaborative study are given in Table 1 for boys from the Philippines. Over an age range from six months to five years there is a fairly constant difference between the tenth and fiftieth percentile of 1.44 cm on the average, and between the fiftieth and ninetieth percentile of 1.45 cm on the average, suggesting a fairly symmetrical but narrow distribution. In fact, 80 per cent of the measurements are in a range of about 3 cm. If one assumes the error in taking the measurement to be in the order of 0.5 cm in both directions, it is rather large in relation to the width of distribution. The narrow distribution of data and the relatively large error would suggest that large groups of children need to be examined to furnish sufficient statistical confidence for a scaled assessment of nutritional status. Objection to the use of circumferences for surveillance purposes has been raised on the grounds that the measurement is beyond the level of skill of auxiliary health workers, that it has insufficient inter- and intra- personal repeatability, and that it is not a sensitive indicator of recent changes in nutritional status (10).

The arm circumference related to height has been used as an indicator in relief operations in Nigeria and Bangladesh for a quick classification of malnourished children. Reference standards were derived from previous local survey data. The method is fast and simple and needs no complicated or heavy equipment. However, the correct measurement of the arm circumference in large surveys requires careful training of field workers and repeated standardization later on (11). The method has been found unsuited for clinical use but to be a useful tool in emergencies. The results in Bangladesh compared well with weight-for-height measurements (11). The sensitivity of this measurement is reduced in a generally less well-nourished population.

Weight for age. The repeated measurement of body weight or total body mass is the simplest, most direct, and most common assessment of growth. It has long been used in pediatrics for assessment of the growth and development of individual children. If a single measurement

TABLE 1. ARM CIRCUMFERENCE, PHILIPPINES, MALES

Age years	Centiles			Mean
	10	50	90	
0.49	12.1	14.0	15.5	13.8
0.74	12.4	13.9	15.5	14.0
1.03	12.4	13.9	15.5	13.9
1.51	12.5	14.0	15.5	14.0
2.04	12.9	14.5	15.9	14.4
2.51	13.2	14.5	15.7	14.5
3.03	13.5	15.0	16.3	14.9
3.54	13.7	15.0	16.5	15.1
4.03	13.8	15.1	16.5	15.1
4.54	13.7	15.0	16.5	15.1
5.09	13.9	15.0	16.5	15.1

Sample sizes 153-327 children per age-group.

Average difference 50th - 10th centile 1.44 cm
90th - 50th centile 1.45 cm

of weight is used to assess the nutritional status, it has to be compared with a reference, the weight of a "normal" child of the same age. Gómez has introduced weight for age for the classification of malnutrition, and this has been widely used and recommended both for individual patients (Morley's growth charts) and for the assessment of the prevalence and severity of malnutrition (1, 13, 14) in communities or populations. The simplest way of classifying a child population is the use of percentages of the reference value as class limits. A more sophisticated procedure is comparison with the distribution of values in the reference population by using percentiles of the reference population as class limits.

Apart from the general difficulty of defining and finding a "normal" reference population without overnutrition or undernutrition, the use of weight for age as a measure of nutritional status has two obvious disadvantages. The first is the fact that in severely malnourished children edema may partly compensate for deficit weight. Such children would thus not be classified as severely malnourished (14). The second reservation concerns the difference in height or body length that may exist between the population surveyed and the reference population. It is well known that malnutrition in children over some length of time produces a deficit in height as well as in weight (15), whereas an acute episode of malnutrition or malnutrition seen in its initial stages has little effect on skeletal growth (14). An assessment by weight for age will therefore overestimate actual malnutrition by including in that figure children that are unusually small for their age (12, 16). It will also overestimate overnutrition by the same mechanism.

Height for age. Height for age, on the other hand, can be used as an indicator of the nutritional status of groups and will estimate past and chronic malnutrition but not necessarily the present nutritional status. It has been shown that the height of African preschool children was related to the socio-economic conditions of their families as well as to intakes of energy, protein, and other nutrients (17). However, there are disadvantages of using only height as an indicator: a deficit in height takes some time to develop; the deficit may not be manifest in infant malnutrition; and

when found in infants or young children it may be the consequence of small size at birth rather than an indication of postnatal malnutrition.

Genetically determined differences are partly responsible for the differences in hight found in any age-group in any population. A preponderance of a genetic trait for large or for small stature in particular ethnic groups has occasionally been shown (18). In most cases, this will be difficult or impossible to prove, but the possibility of ethnic differences in height cannot be completely discounted.

Weight for height. In order to eliminate the influence of height when comparing weight with a reference standard the weight found at a certain age is often corrected for height by comparing it with the weight of a reference group of the same height rather than of the same age (1, 12, 19, 20, 21). An indicator of leanness or thinness and fullness is thus obtained. Since the commonly used reference standards for weight and height do not give the distribution of weights over height at different ages, weight-for-height can be expressed as a percentage of the reference median weight for median height at each age. One obvious advantage of using weight-for-height as an index of nutritional status is its apparent age independence (21). This is of special significance and usefulness in areas where ages are not recorded and often not known. Whenever ages are known it is desirable to obtain, in addition to weight-for-height or degree of thinness, information on growth performance, i.e., height-for-age (14) -- in other words, information on past as well as present nutritional status.

In order to combine the two types of information on a population a classification in a four-by-four tabulation has been proposed (22) in which children are grouped horizontally by per cent weight-for-height or the nutritional present, and vertically by per cent height-for-age or, in a sense, the nutritional past. Depending on the purpose for which the classification is intended, the width of the classes can be varied. The simplest form suggested is a two-by-two table which divides children by four criteria (above or below 80 per cent weight-for-height and above and below 90 per cent height-for-age) into four categories to which different priorities for action can be assigned (21), but which can also serve for monitoring purposes. An example is given below:

Wasted and stunted	Priority
Wasted but not stunted	Action
Not wasted but stunted	Action?
Not wasted and not stunted	No action

The priorities given to the four categories must be decided upon in the light of local circumstances, since the conditions they represent are likely to be caused by different agents. In a wasted and stunted child population the possibility of ethnic and non-nutritional growth limitations should be excluded. Wasting without stunting would be expected in a population hit by an emergency, stunting without wasting may be due to earlier non-nutritional influences (23) and/or to previous prenatal or postnatal malnutrition. The identification of the main causative factors is important for deciding to take action as well as for the choice of the most suitable and promising type of action.

Another consideration that should be taken into account concerns the interpretation of height deficits. Obviously the significance of stunting, if expressed as a percentage of a reference height, changes with age. The normal increase in length is small in relation to the length at birth, a normal child taking three to four years to double its length. Early stunting thus may have a different meaning from the same degree of stunting in later childhood. The interpretation is complicated by the normal age dependence of growth velocity, the usually unknown length deficit that may exist at birth, and the catch-up growth that has been observed when conditions improve (24).

The indices that emerge as the most promising and practical from this analysis are: a) per cent weight-for-age; b) per cent weight-for-height; c) per cent weight-for-height combined with per cent height-for-age.

Weight-for-age, in spite of its lack of specificity, has the advantage of being the

simplest to measure. It is already widely used as a rough measure of malnutrition in populations (13) and for the follow-up of individual children. When growth charts based on weight-for-age have been introduced in clinics and services for mothers and children, information on the distribution of weight-for-age is relatively easily available for nationwide estimates of the nutritional status of small children, but the sample is not strictly representative. For the assessment and monitoring of the nutritional status of a child population weight-for-age data are particularly valuable in infancy, a period during which the measurement of length is technically difficult and at which the retardation in growth is relatively small and insignificant in many populations. For the purpose of screening of a population for cases of malnutrition this index is clearly less useful. Although cases of clinical malnutrition usually exhibit a very low weight-for-age index, for the reasons discussed above, this index would be expected also to classify as malnourished a considerable number of small but healthy children, particularly in later preschool age, and children with less severe degrees of malnutrition.

Weight-for-height has the advantage of a clearly defined meaning which is easily understandable, measuring as it does the thinness or fullness of the body. Its age independence adds to its ease of use and also allows its use in populations where ages are uncertain or not known. Measurements of the food consumption of children recovering from severe protein-energy malnutrition in Jamaica have shown that the high food intakes during recovery were spontaneously reduced to normal as soon as a normal weight-for-height was reached (25). This observation was confirmed in a still unpublished study on about 50 children in Mexico (J. Cravioto) as well as in experimental feeding of healthy infants in India (26) and may be taken as an indication that the relationship between weight and height in well-fed children is an expression of a biological constant which is somehow involved in the regulation of food intake.

There are obvious disadvantages and limitations to the use of weight-for-height. One is the difficulty of measuring body length in young infants, which may make it difficult or sometimes impossible to obtain adequate data in this age-group. Another is inherent in its being a specific expression of thinness-fullness: it gives no information on the duration of malnutrition or of past malnutrition.

Standards of reference. The use of anthropometric data for the assessment of nutritional status depends, for most purposes, upon the availability of adequate standards of reference. Exceptions are the follow-up of individual children, e.g., on a "growth chart", and the monitoring of changes in a population for purposes where no value judgement nor community diagnosis is needed. Usually, however, a true assessment will be a necessary component of a nutritional surveillance. In this case a standard of reference is needed for comparison. A case has been made for the use of internal or local standards to be developed from the growth performance of well-to-do and presumably well-fed children living in the same area (1,22), based on the assumption that genetic or ethnic differences in growth potential are a significant determining factor in the growth of children in different populations (22). However, a recent review of available evidence has shown that ethnic differences play a minor role in the actual growth performance of populations if compared with the overwhelming influence of nutrition and disease (30). There seems to be good justification for the use of reference data from western populations which, if only because of the lack of adequate local standards of reference, are widely applied already. However, since the possibility of true ethnic differences cannot be completely ruled out at present, caution should be observed in the interpretation of comparative data, particularly if differences are small. This would apply especially for age-dependent indicators like weight-for-age and height-for-age.

Treatment of data. The way in which the data are processed, compared, and presented will depend upon the scope of the survey or surveillance but also on the available facilities for data collection and handling.

The problem of comparing the distribution of the data from the sample with the distribution in a reference population is often overcome by the use of a more or less arbitrary cut-off point and the percentage of values below or above it, thus providing a single figure to decision makers. For the monitoring of general nutritional trends it may be possible to choose cut-off points according to the expected sample sizes and distributions in such a way as to ensure proportions that make statistically significant statements possible.

For screening for individual health care, supplementary feeding, or relief operations, cut-off points serve for the identification of individuals who should be treated with priority. In the case of the weight-for-height index discussed above, the cut-off point usually recommended is 80 per cent of the reference weight-for-height on the basis of clinical observations. The operational practice of a health or nutrition program, however, may make it necessary to choose a lower cut-off point in order to remain within the limits for services set by the available resources.

When surveillance systems are established under adverse conditions they may have to rely upon data that are already available or are being collected routinely by existing systems. Such data are often from small samples or are incompletely reported in summary form. In such cases the only possible way to make use of the data may be the comparison of the means or medians, whichever are reported, with the reference mean or median. Weights and heights of children are often routinely taken and recorded in clinics, mother-and-child-care centers, and schools, and are reported separately. Average or median weight-for-height figures can easily be calculated from such data and used, with the necessary caution, for an assessment of the nutritional status of such groups. This will not yield quantitative data on the degree of malnutrition but can give valuable indications of it.

In national food and nutrition policies nutritional surveillance has the important function of monitoring the success of policies and programs and of any modifications of them that may be necessary. Many of those policy measures may be in the socioeconomic field - in production, marketing, price, policies, and income distribution. The effect on malnutrition in preschool children will be indirect because in addition to the availability of food, cultural and environmental factors are important in its causation. Preschool child malnutrition may thus not react immediately to change but rather with a time lag of unknown length, and other age-groups may well be more sensitive indicators of change. There is still very scanty evidence of such relationships except on an international scale including the most affluent and the poorest countries, where preschool child malnutrition is clearly related to poverty (2, 13). However, extensive cross-sectional surveys in rural and urban areas in India have shown that the average food intake of preschool children is not associated or only slightly associated with per capita income, food availability in the family, or adequacy of the family diet (27, 28). In contrast the food intakes of older children and of adults rise significantly with per capita income and food availability. These findings are confirmed by an in-depth study of a small group in the Punjab (29) in which the income elasticity of consumption for calories in the age-group 18 to 24 months was only 0.082, meaning that a doubling of the family income would increase the child's calorie intake by only 8 per cent, while the percentage increase in the calorie intakes of adults would be about ten times as large. The same investigators found no change in the average percentage weight-for-height with increasing per capita income except for a significantly lower value in the lowest income group (below the equivalent of US $3 per month).

Information of this kind seems at present not to be available from other countries, and in view of the distinct cultural differences between India and other parts of the world it would be dangerous to generalize from such reports without any supporting evidence. But the Indian findings draw attention to the need to include other age-groups in the search for indicators.

Older children and adults

After the preschool age clinical malnutrition is only exceptionally seen, but nutritional status as assessed by weight-for-height is not necessarily improved. The Singapore Indians continue to have a very high proportion of thin children. The values are much higher than in any other group and one may be tempted to see this as an expression of an ethnic characteristic (35). But there is no reason to assume that the group is ethnically uniform. The term Indian refers to the common culture of the Indian subcontinent and cannot be taken as an ethnic classification in a country with a history of several thousand years of foreign invasions and much ethnic admixture. In fact, the mean weight-for-height figures of Singapore Indians compare well with those of a growth study that was carried out in different parts of India and represents a cross-section of its ethnic composition (31). Captive groups school-children are easy to reach, a fact that is of considerable practical importance for a regular flow of information. One has to take into consideration that, where schooling is not yet universal, the children attending school are probably a biased sample of the population. This will not make the data useless and, unless the bias changes, schoolchildren may still be a valuable index population for the measurement of change.

The onset of adolescence is characterized by a spurt in bodily growth together with growth of the reproductive organs and numerous physiological changes in both sexes. The time at which the growth spurt sets in and its intensity are obviously influenced by nutrition, but nutrition is only one of several factors (32). Because of the complexity of the changes occurring at this age, adolescence does not seem to be an age period particularly suited for nutritional surveillance.

With sexual maturity individual differences in body composition increase. In males the development of musculature becomes an important variable influenced by androgenous hormones, physical training, and nutrition. In western adult populations there is an increase in body weight with age, which appears to result from a gradual increase of body fat and a concomitant decrease of lean body mass (33, 34) that seems to reach its maximum between twenty-five and thirty years of age. This pattern is possibly characteristic of affluent populations with abundant food supply and little physical activity. Table 2 gives the body weight of adults from a number of Latin American and Asian countries with varying food supplies (35, 36) showing a different pattern in most countries. A detailed study from the New Guinea highlands has shown that the observed decrease of weight with age is accompanied by a gradual decrease of skinfold thickness, of cross-sectional limb muscle areas and of creatinine excretion (36).

For the assessment of nutritional status and its changes in adults the changes in several body compartments should be taken into account. At least some measurement of body fat such as skinfold thickness should complement weight and height data. A reasonably close relationship between skinfold thickness at various sites and body density has been established experimentally and allows the calculation of body fat in per cent of body weight from single measurements at different adult ages (34). This approach has not yet been applied to large populations and needs further testing.

The importance of an assessment of nutritional status in adults for the purposes of nutritional surveillance is not yet very clear, and its methods have so far received relatively little attention. Yet adults are not only the main consumers but also the main producers of food and in non-mechanized agriculture the nutritional status of farm labor may well influence output (37). In urban populations industrial workers are often a relatively uniform group that is often more accessible than other parts of the population. Given the high proportion of earnings spent on food in most populations with a low income, this group could be expected to reflect fluctuations in purchasing power in its nutritional status.

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TABLE 2. CHANGES IN ADULT BODY WEIGHT WITH AGE (35, 36)

Country	Weight at 25-29 years kg	Weight at 50-55 years kg
Uruguay	70.0	76.0
Chile	64.3	69.0
Venezuela	62.4	66.8
Bolivia	61.0	63.0
Colombia	59.6	55.2
NE Brazil	58.0	57.2
Ecuador	57.5	57.5
Japan	56.7	56.0
Thailand	53.0	51.8
West Pakistan	52.6	56.2
Malaysia	52.2	52.7
Viet-Nam	49.0	47.4
East Pakistan	45.6*	45.0*
New Guinea	49.7*	41.6*

*Calculated from regression.

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INFORMATION BEARING ON THE EVALUATION OF THE HAZARD TO MAN FROM AFLATOXIN INGESTION

Leonard Stoloff and Leonard Friedman*

Although there are differences in species susceptibility to aflatoxin carcinogenesis (Wogan, 1973), most projections of the possible hazard from ingestion of aflatoxin by man are derived from experimental results (Wogan et al., 1974) based on studies with male Fischer strain rats fed a synthetic diet in which crystalline aflatoxin B_1 was incorporated at various levels from 1 to 100 ppb. Liver carcinomas were detected in 2 of the 22 rats on the 1 ppb diet for 104 weeks (2/3 of average life span), and in 28 of the 28 rats on the 100 ppb diet for 54 weeks. A number of questions need to be answered before the translation of these data to man can be considered valid. In answering the questions, other approaches to the assessment of a possible hazard to man from aflatoxin ingestion may be developed.

Question 1. - Does the Fisher rat strain have unusual sensitivity to aflatoxin caricinogenesis?

This question was raised by studies (Alfin-Slater et al., 1969) with USC rats that had no tumor development in any organ after 90 weeks on a natural diet containing aflatoxin B_1 at 53 ppb plus aflatoxins B_2, G_1 and G_2 at 31, 36 and 28 ppb, respectively. The source of the aflatoxins was naturally contaminated peanut butter. A lifetime feeding study (Ness et al., 1975) to resolve the observed difference found that the Fischer rat strain developed liver tumors with mixed aflatoxins ($B_1 : B_2 : G_1 : G_2 = 3 : 0.9 : 2 : 0.6$) at 5 ppb total aflatoxins in the diet, whether introduced as crystalline aflatoxins or as a natural contaminant, and whether the diet was synthetic or natural. The difference in observation is, by elimination, due to the strain of rat.

In a later study (Nixon et al., 1974), Fischer strain rats were compared with Wistar strain rats after 2 years on a semipurified diet containing 20 ppb aflatoxin B_1. There were no hepatomas in the Wistar rats compared to a 21 per cent incidence in the Fischer rats. When Wistar rats were fed 100 ppb aflatoxin B_1 in the diet, there was a 42 per cent incidence of hepatomas; Fischer rats in this study received no similar high dose, but from past studies with this strain in another laboratory (Wogan et al., 1974; Wogan and Newberne, 1967) a 100 per cent incidence would have been expected. Summation: Compared to USC and Wistar strain rats, the Fischer strain rat is highly sensitive to aflatoxin carcinogenesis.

Question 2. - Is aflatoxin carcinogenic to resistant animals at any level?

*Bureau of Foods, Food and Drug Administration, Washington, D.C. 20204.

Mice are resistant to aflatoxin carcinogenesis under conditions that result in 100 per cent tumor incidence in Fischer strain rats. However, a single i.p. dose of aflatoxin B_1 in trioctanoin resulted in hepatomas in 5/22 newborn mice (C57BL x C3H F1, both sexes) in 82 weeks (Vesselinovitch et al., 1972) and in 55 per cent mortality. The dose of 2 µg/g body weight translates (assuming a 2 g mouse consumed 0.2 g of feed/day) to approximately 20 ppm aflatoxin B_1 in the feed, if taken orally. In an associated experiment, 21 of 38 mice (both sexes) developed hepatomas after being dosed i.p. with aflatoxin B_1 at 0.25 µg/kg body weight 5 times at 3-day intervals, starting at 4 days of age. This dose, which caused no acute symptoms, translates to approximately 3 ppm aflatoxin B_1 in the food, if taken orally. Male White Swiss mice (Louria et al., 1974), weighing 20 g at the start of the experiment, were dosed 5 times weekly by intubation with 100 µg of mixed aflatoxins ($B_1 : B_2 : G_1 : G_2$ = 40 : 5: 50 : 5) suspended in water. After 1 year (1/2 of average life span) these mice showed no liver damage but the possibility of an enhanced incidence of an endemic leukemia. The dose translates (assuming 3 g of feed consumed per day) to approximately 15 ppm aflatoxin B_1 in the feed.

The limited number of nonhuman primates studied, 170 animals in 12 different reports, were relatively resistant to aflatoxin carcinogenesis compared to the Fischer rat. In one study (Lin et al., 1974), 1 of 9 marmosets developed a liver tumor after 50 weeks (1/11 of average life span) on a diet containing 2 ppm aflatoxin B_1, 5 days each week. The animal was a male in a mixed sex group. The authors noted that liver damage in the marmoset included cirrhosis, which is not a symptom of aflatoxicosis in the rat. In another study (Reddy et al., 1976), 9 of 18 tree shrews getting 2 ppm aflatoxin B_1 in their chow approximately half the time on an on-off schedule, developed liver tumors between 74 and 172 weeks (a full average life span) on the diet. The animals were of both sexes. Females incurred a higher incidence of tumors than males, the reverse of the usual pattern in other animals. In a third study with 2 monkeys (Gopalan et al., 1972), a male Rhesus monkey given a daily dose of mixed crystalline aflatoxins in water for 5.5 years, introduced intramuscularly during the first year, develop a hepatoma after 7.5 years. The estimated daily dose of aflatoxin B_1 was 20 µg/kg body weight, which translates (assuming a 5 kg animal consumed 250 g of feed per day) to approximately 400 ppb aflatoxin B_1 in the feed. The second monkey (female) dosed at half this amount developed an intrahepatic cholangiocarcinoma after 11 years (Tilak, 1975). This lesion is not typical of aflatoxin-induced hepatocarcinogenesis in the rat (Newberne and Butler, 1969). In a fourth study (Adamson et al., 1973), two Rhesus monkeys (1 male, 1 female) developed liver tumors after being dosed with crystalline aflatoxin B_1 by various routes at an estimated average level of 60 µg/kg body weight for 5.8 years. This translates to approximately 1.2 ppm aflatoxin B_1 in the feed. The remaining 38 monkeys of both sexes in this test group show no evidence of tumors after 9 years (3/5 of average life span) of being dosed with an estimated 0.5 ppm aflatoxin B_1 calculated from food intake. Liver tumors do not often occur spontaneously in monkeys (O'Hara, 1972).

Summation: Liver tumors can be induced in mice by a dose in the ppm range (level translated to feed) by the i.p. route at a very early stage of life. A tumorigenic response was induced in a subhuman primate by the equivalent of 0.2 ppm aflatoxin B_1 in the feed. The type of tumor produced in the subject animal at this dose was not typical of liver tumors associated with aflatoxin carcinogenesis in the rat in addition, the administration of the toxin included the intramuscular route.

Question 3. - Is the liver the only target organ for aflatoxin carcinogenesis?

Although the liver is the primary target organ in most situations, cancerous lesions have been observed in other tissues in some animals challenged with aflatoxins.

Kidney. - In one study (Butler et al., 1969), MRC rats of both sexes were given either crystalline B_1 or G_1 in their daily drinking water for 20 weeks. The levels, calculated on the basis of feed from normal rat intake

(assuming a 400 g rat consumed 20 g of feed per day), were 4 and 1.3 ppm of G_1 and 1.3 ppm of B_1. All groups developed a high incidence of hepatic tumors, but renal tumors (adenomas) were found only in the male rats. The published conclusion that the renal tumors were benign was not shared by all pathologists who examined the tissues (Wogan, 1975). Renal tumors were found in 2 of 15 male rats challenged with aflatoxin B_1 after 71 and 72 weeks; renal tumors were found between 54 and 78 weeks in 6 of 11 male rats challenged with 4 ppm G_1 and 5 of 15 male rats challenged with 1.3 ppm G_1. Occurrence of hepatic and renal tumors was not coincidental in all animals.

In another study (Epstein et al., 1969), crystalline aflatoxin B_1 incorporated in the diet at levels of 1.0, 0.5, and 0.25 ppm, was administered to male Wistar rats for 147 days. Renal tumors (adenomas) developed at all three levels at dose-related rates of 8/14, 5/18, and 3/13 and dose-related mean autopsy times of 603, 696, and 783 days. A high incidence of hepatic tumors was also seen, but occurrence of renal and hepatic tumors was not coincidental in all animals.

Yet another strain of rat, the Long-Evans, developed a 70 per cent incidence of renal tumors (adenomas) after 18 months on a diet containing 300 ppb aflatoxin B_1 and 252 ppb aflatoxin G_1 (Lee et al., 1969).

In an earlier study (Butler and Barnes, 1968) with the Porton strain of rats on a diet containing aflatoxin-contaminated peanut meal, renal adenocarcinomas were found in 5 of 53 male rats exposed to 0.1 or 0.5 ppm in the diet over the entire life span.

Colon. - In a study of the effect of marginal dietary vitamin A on aflatoxin carcinogenesis in the male rat (Newberne and Rogers, 1973), colon carcinomas were observed in 6 of 50 rats of the Charles River strain on a semi-synthetic diet containing 100 ppb crystalline aflatoxin B_1, and in 1 of 20 Fischer strain rats on the same dietary regimen. Other observations included a colon carcinoma in 1 of 25 Fischer strain rats on a diet containing 15 ppb aflatoxin B_1, and in 1 of 15 female Fischer and 2 of 49 male Charles River rats administered the equivalent of a dietary level of approximately 4 ppm aflatoxin B_1 by intubation in 10 to 15 daily doses. A high incidence of hepatic tumors was also observed, but the occurrence of hepatic and colon tumors was not coincidental in all animals.

Lung. - Combined feeding of urethan (ethyl carbamate) and aflatoxin (Newberne et al., 1967) to male rats of the Charles River strain resulted in a lower incidence of hepatic tumors than when urethan was not included. However, lung adenoma and malignant lymphoma, respectively, were seen in 1 and 3 of 20 rats after an average 320 days on a diet containing 0.4 ppm aflatoxins from contaminated peanut meal and 0.2 per cent urethan. An increase of the aflatoxins to 1.5 ppm and urethan to 0.4 per cent for an average 349 days produced 1 lung adenoma and 2 malignant lymphomas in 25 rats. No lung or lymph lesions were seen in animals receiving aflatoxin without urethan, but malignant lymphoma and hemangioendothelioma of the lungs were seen in rats, receiving 0.2 per cent urethan without aflatoxins. Pulmonary tumors (Wieder et al., 1968) were seen in 14 surviving female strain A/He mice (a strain subject to a high incidence of spontaneous lung tumors) 20 weeks after receiving crystalline aflatoxin in DMSO by the i.p. route, 3 times each week for 4 weeks. Each injection contained 20 µg aflatoxin B_1, which translates (assuming 21 g of feed/week) to approximately 3 ppm of aflatoxin B_1 in the feed intake.

Harderian Gland. - Adenoma of a lacrimal gland (Herrold, 1969) was observed in 1 of 10 Syrian hamsters of both sexes receiving 0.1 mg of mixed aflatoxins by intubation twice weekly for 10-11 months, and in 3 of 10 hamsters receiving 0.2 mg mixed aflatoxins, i.p., once each week for 6-8.5 months. Observations were made at 12-30 months on the first group and at 7-22 months on the second. The aflatoxin composition was $B_1 : B_2 : G_1 : G_2 = 4 : 1 : 4 : 1$. Both routes caused lesions of the liver, kidney, small intestine, periodontal membrane and lacrimal gland, but identifiable tumors only in the lacrimal gland. The challenge translates (assuming 70 g of feed/week) to approximately 3 ppm aflatoxin B_1 based on the feed.

Lacrimal gland carcinomas (Goodall and Butler, 1969) were seen in 4/14 hypophysectomized rats after 35 weeks on a diet containing 4 ppm total aflatoxins (B_1 : G_2 : G_1 : G_2 = 7 : 1 : 7 : 1) from contaminated peanut meal.

Summation: The possibility of aflatoxin induced tumors of the kidney, colon, lung and lacrimal gland has been demonstrated. The case for lung tumors is weak, being based on a strain of mice subject to a high incidence of spontaneous lung tumors and on either a simultaneous challenge with urethan or massive i.p. administration in DMSO. Dose levels required to induce tumors of the other organs were, in most cases, in the ppm range. There was a difference of opinion between pathologists who examined the renal tumors as to state of malignancy. Variable species susceptibility to each type of tumor is suggested by the experimental results, but the data are insufficient for even a tentative conclusion.

Question 4. - Is there an increased risk during the prepartum or preweaning periods?

Malformations were induced in the offspring of of hamsters (Elis and DiPaolo, 1967) a administered aflatoxin B_1 by the i.p. route on day 8 of pregnancy at a level of 4 mg per kg body weight. No malformations were seen when the dose was 2 mg per kg. The 2 mg translates (assuming a 400 g hamster consumed 20 g of feed/day) to approximately 40 ppm of aflatoxin B_1 in the feed. Malformations could not be induced in the offspring of rats or mice administered higher doses than those used for the hamsters (DiPaolo et al., 1967).

In another study (Grice et al., 1973), female Wistar rats were given a diet containing aflatoxin-contaminated peanut meal that provided aflatoxin B_1 levels of 2.5 and 5 ppm. The animals were on the toxic feed according to 3 different schedules: day 10 of pregnancy to parturition, 1 day postpartum to 10 days postpartum, day 10 of pregnancy to 10 days postpartum. Liver carcinomas were found in 4 of 208 offspring with no relation to dose level or regimen. Lesions of the liver and other organs were observed at a much higher incidence in the offspring of dams receiving aflatoxin than in the offspring of controls. Of 208 offspring from treated dams that were examined, 4 had malignant liver tumors and 39 had other liver lesions; no similar lesions were seen in 65 control progeny.

Summation: The limited information with one species (hamster) showed that a relatively high single dose caused terata. No delivered terata were seen in a study with rats dosed over the entire vulnerable period, but this last study did show some danger of oncogenesis from exposure through the placenta or milk from these high levels.

Question 5. - Is aflatoxin per se the carcinogen?

In their resistance to aflatoxin carcinogenesis, animals vary between species and between strains of the same species (see questions 1 and 2). Within a susceptible species and strain there is a marked sex difference in response to an aflatoxin challenge (Wogan and Newberne 1967). This difference can be related to the sex hormones (Newberne and Williams, 1969; Cardeilhac and Nair, 1973). Removal of the pituitary gland (Goodall and Butler, 1969), protein deprivation (Madhaven and Gopalan, 1968), and administration of phenobarbitone (McLean and Marshall, 1971), treatments known to alter the activity of microsomal enzymes of the rat liver, also imparted resistance to aflatoxin carcinogenesis in male rats.

That reduced carcinogenesis following these treatments is related to modified microsomal enzyme activity may be deduced from the following evidence:

1. There is a strong theoretical and experimental basis for metabolic activation of many chemical carcinogens and for their action by alteration of the informational content of chromosomes (Miller and Miller, 1966).

2. In vivo administration of aflatoxin B_1 to rats strongly inhibits RNA synthesis in the rat liver (Moulé and Frayssinet, 1972; Friedman and Yin, 1973; Neal, 1972). This inhibition is not evidenced when aflatoxin B_1 is added to an in vitro cell-free system (Moulé and Frayssinet, 1972; Sarasin and Moulé, 1973;

Belt and Campbell, 1975), except when chromatin, rather than DNA, is used as the template (Portman and Campbell, 1970). Rat liver microsomal metabolites of aflatoxin B_1 do cause *in vitro* inhibition of RNA synthesis (Sarasin and Moulé, 1973; Belt and Campbell, 1975); whether chromatin contains aflatoxin-metabolizing enzymes has not been established. Removal of the rat pituitary gland (Friedman and Yin, 1973) or pretreatment with phenobarbitone (Neal, 1972) prevents the *in vitro* inhibition of RNA synthesis observed with *in vivo* administration of aflatoxin B_1. Removal of the pituitary also alters the observed pattern of aflatoxin metabolites produced by the nonmicrosomal liver fraction.

Evidence is accumulating that one of the active metabolites may be aflatoxin B_1-2, 3-oxide (Garner, 1973; Swenson *et al.*, 1975, 1974 and 1973), but because of its probable lability, no direct determination of its presence has yet been made.

Summation: There is good inferential evidence that a liver metabolite(s) of aflatoxin B_1 is the active carcinogen in the rat.

Question 6. - Are there any differences in the metabolism of aflatoxin by livers of animals resistant and susceptible to aflatoxin carcinogenesis and how do these differences relate to man?

In responding to this question, the temptation to identify specific metabolites as part of the elimination or of the oncogenic pathways will be avoided, since, at the present state of knowledge, such identification is highly speculative. The first attempts at comparing the *in vitro* metabolism of aflatoxin B_1 by livers or liver microsomal preparations of different species has been reviewed by the principal investigator (Patterson, 1973). Although these first studies suffered from a limited knowledge of ultimate metabolites and lack of radiolabeled aflatoxins, a number of unequivocal observations could be made:

1. An LD_{50} dose of aflatoxin B_1 is undetectable in a matter of minutes when incubated with liver preparations (9000 x g supernatants) of rabbit, duckling, and guinea pig, in a matter of hours when incubated with liver preparations of chick, mouse, pig, and sheep, and in a matter of days when incubated with a rat (unidentified strain) liver preparation.

2. The pattern of chloroform-soluble *in vitro* metabolites varies from species to species when mouse, rat, rabbit, guinea pig, chick, duck, quail, turkey, cattle, goat, pig, and sheep livers are compared.

3. Phenobarbitone stimulation of *in vitro* microsomal metabolism enhanced production of aflatoxins M_1 and P_1 in the rat, but not in the duck.

Subsequent comparisons of aflatoxin metabolism by rats (Wistar derived strain) and mice *in vivo* (Steyn *et al.*, 1971) and rat and mouse liver preparations *in vitro* (Steyn *et al.*, 1971; Faris and Hayes, 1975; Portman *et al.*, 1968), using ^{14}C ring-labelled aflatoxin B_1, confirmed the much slower metabolism by the the rat and the big difference in type and quantity of metabolites found. But in another experiment (Roebuck, 1975) the rat and mouse liver preparations were found to be equally slow compared to the duck, monkey and man.

Aflatoxin Q_1 (Roebuck, 1975; Masri *et al.*, 1974b; Steyn *et al.*, 1974; Buchi *et al.*, 1974) was a major metabolite produced *in vitro* by livers of monkey and man and a very minor metabolite of the rat liver. Aflatoxin P_1 (Dalezios *et al.*, 1971) was a major product, in relation to other known products of metabolism of aflatoxin B_1 by monkey liver *in vitro* and *in vivo* (Roebuck, 1975; Dalezios, 1971) and human liver *in vitro* (Roebuck, 1975, Dalezios *et al.*, 1971; Merrill and Campbell, 1974); this compound has not been detected as a metabolite of any of the other species. Because the quantity of aflatoxin P_1 produced is different when aflatoxin B_1 is administered *per os* than when it is given i.p. (Dalezios *et al.*, 1973), its significance as a metabolite is currently in question. The duck liver converted nearly all metabolic products to water-soluble form. The major *in vitro* conversion of aflatoxin B_1 to Q_1 by monkey liver was confirmed in another experiment (Masri *et al.*, 1974a), in which aflatoxin P could not be found as a

monkey liver metabolite. The higher rate of B_1 metabolism in monkey liver compared to rat liver preparation was also confirmed in this experiment; an average 55 per cent (three monkey species) of the metabolized B_1 was converted to Q_1 by monkey livers compared to 3 per cent by rat livers. The rats used were from a strain that was demonstrated to be susceptible to aflatoxin carcinogenesis. Aflatoxin M_1 was a minor metabolite of both monkey and rat livers: 0.6 per cent of metabolized B_1 by monkey liver, and 1.7 per cent of metabolized B_1 by rat liver. Pretreatment of the rats with phenobarbitone doubled the rate of aflatoxin metabolism by rat liver microsomes and increased to only 5 per cent the proportion of Q_1 to metabolized B_1 without increasing the proportion of M_1 found. This observation is also relevant to the discussion of Question 5.

Another in vitro metabolite, aflatoxicol H_1, has been identified as common to livers of monkey and man (Salhab and Hsieh, 1975), but its identification is too recent for possible detection as a metabolite of other species.

Summation: In vitro experiments, although in some cases producing contradictory results, show major differences in rate of aflatoxin metabolism and the pattern of metabolic products formed by liver preparations of the various species studied. The aflatoxin metabolism pattern of human liver is generally different from that of the rat and most nearly resembles that of monkeys. In this last regard refer to the response to Question 2.

Question 7. - Is there any epidemiological evidence relating to the susceptibility of man to aflatoxin carcinogenesis?

A review (Campbell and Stoloff, 1974) of four epidemiological studies in different areas of the world, where both the incidence of liver cancer and contamination of foodstuffs with aflatoxins are relatively high, shows a general correlation between the current exposure of the populations to aflatoxins and the level of liver cancer reported. The same observation was made in a report (Van Rensburg et al., 1974), published since the review, of a study in a fifth area. There are three major caveats in using this type of evidence: 1) Correlation should not be confused with causation. 2) The long induction period for liver cancer (peak incidence around age 45 in the areas studied) requires the assumption of a stable population and aflatoxin exposure pattern. 3) The individual exposure to aflatoxins cannot be obtained from the total of aflatoxins in the foods analyzed averaged over the population at risk, as assumed in these studies. 4) The possibility exists that the real incidence of cancer may be underestimated because of deficiencies in health care facilities in underdeveloped countries.

Three of the studies (Campbell and Stoloff, 1974 (Kenya, Thailand); Van Rensburg et al., 1974) are based on aflatoxin analyses of typical family meals. The liver cancer rates from these studies appear to fit a relation with the log of the average aflatoxin intake. The last study provides 2 cancer rates, based on different data bases and on the aflatoxin exposure as total aflatoxins, compared to the use of B_1 only in the other 2 studies. Use of the lower cancer rate and the assumption that total aflatoxins equate to aflatoxin B_1 would be a conservative application of these data to a risk analysis. The seven points obtained from these studies provide a regression line (cancer rate = $-3.96 + 8.42$ log aflatoxin exposure, $p < 0.01$) which produces a zero rate at an aflatoxin B_1 exposure of 3.0 ng/kg body weight/day. The calculation contains other conservative features in that it does not allow for the possibility of liver cancer from other causes nor for the consumption of aflatoxin-contaminated foods outside of the family meals. Evidence from one study (Campbell and Stoloff, 1974 (Thailand)) is that this exposure to aflatoxin could be as great for all the populations observed as the worst cookpot situation. On the assumption that normal food intake is about 25 g/kg body weight, the 3.0 ng figure can be calculated to 0.12 ppb of the food consumed.

Summation: All 5 studies of aflatoxin ingestion in areas having local populations with relatively high rates of liver cancer show a positive correlation, but provide no basis for a conclusion that aflatoxin is a causative factor. On the assumption that aflatoxin is a causative

factor, the data provide a conservative estimate that in a population exposed to an average 0.12 ppb aflatoxin B_1 in its food, the risk of liver cancer is statistically very low.

Question 8. - Are there any other known causes of liver cancer that could affect the epidemiological findings?

The distribution and etiology of primary hepatoma have been thoroughly reviewed (Burdette, 1965). Natural products demonstrated to be hepatocarcinogenic in test animals include: the mold toxin sterigmatocystine (Purchase and Watt, 1970); mold toxins associated with yellow rice disease; ethionine, produced by some bacteria; cycasin, from cycad nuts; pyrrolizidine alkaloids, from the Senecio, Crotolaria, and Heliotropium genera; saffrole, found in many spices; thiourea, reported to occur in seeds of Laburnum; and selenium compounds, which, dependent on soil levels and conditions, occur in plants. The possible contribution of viral hepatitis or parasitic disease has been considered; although no evidence has been found to link these factors to hepatoma, this remains an unsettled issued. Chronic alcoholism, and the accompanying cirrhosis, was associated with hepatoma in a study at Boston City Hospital (Purtilo and Gottlieb, 1973).

Summation: The populations included in the epidemiological studies could have been exposed to hepatocarcinogens other than aflatoxins, particularly pyrrolizidine alkaloids and selenium compounds, and to alcohol. None of the studies report an attempt to check for possible etiological factors other than aflatoxins, except for one study (Purchase and Goncalves, 1971) in which the foodstuffs were analyzed for sterigmatocystin with negative results.

Question 9. - Does the picture of liver, kidney or colon cancer in the United States indicate any relation to aflatoxin exposure?

Chronic exposure to aflatoxin is most likely to have occurred in the peanut-growing, rural areas of the southeastern states where corn grits is a dietary staple and unmarketable peanuts are likely used for local consumption. FDA (Stoloff, et al., 1976) and USDA (Shotwell et al., 1973; Lillehoj et al., 1975) surveys have shown that aflatoxin contamination of corn is a problem primarily in the southeastern states and that most of this contamination is likely of field origin. The history of corn agronomy and the veterinary history of moldy corn toxicosis in the southeastern states indicate that aflatoxin in corn has probably been endemic in that area. From the survey data, an estimate can be made that the average aflatoxin B_1 contamination level of all corn in the Southeast could be around 20 ppb. The rural South can be different from the urban South in the source of the corn used and the inclusion of the corn germ in the grits (Mayberry, 1975). Rural consumption is most likely to be of local origin and include the germ. Urban consumption is more likely to come from large millers who obtain corn from a wider area beyond the South and whose production is mostly degermed. Removal of the germ can reduce the aflatoxin level of the flour to about 10 per cent of the original corn (Brekke et al., 1975). On the basis of corn grits alone, assuming an average consumption of 50 g/day for a 25 kg child, the daily aflatoxin exposure could be 40 ng/kg body weight.

In Table 1 are listed rates of primary liver cancer from available cancer registries that allow a comparison of rural South vs. urban South and South vs. other U.S. areas.

To decrease the possibility that the rates may be influenced by industrial exposure to carcinogens, the age-specific rates were calculated, where possible, for the age groups in which aflatoxin-induced liver cancer would be expected (Table 2). This expectation is based on the induction period from animal studies and the age for peak incidence of liver cancer in the Asian and African populations exposed to aflatoxins in their foods. Industrial exposure and drug or alcohol addiction, occurring late in life, should not mask effects from food consumption started early in life. The selected age-specific rates do not, however, remove the possibility of contributions by air and water pollutants.

U.S. cancer mortality data by county for 1950

through 1969 (Mason and McKay, 1974) and for 1970 through 1973 (Mason, 1975) show that cancer of the colon and of the kidney occur at the highest rate in the Midwest and Northeast and at the lowest rate in the Southeast. The 1950-69 study does not separate cancer of the bile duct from cancer of the liver, but the 1970-1973 study does make the separation. The data from the latter study confirm the picture derived from the separate registers.

Summation: The data show a negative correlation between the expected exposure to aflatoxin and the incidence of liver, colon, or kidney cancer in the United States, but other etiological considerations cannot be completely eliminated as factors complicating the determination of a relationship.

TABLE 1

Incidence of primary liver cancer from state cancer registers - Comparison of rural and urban southeast with other areas of the United States

Area	Reference	No. yrs. covered	Crude rate/10^5/yr.					
			White		Negro		Other	Total population
			Male	Female	Male	Female		
Alabama			0.96	0.75	1.92	0.68	-	0.97
Rural only	LaVallee, 1972	13-1/2	0.81	0.56	1.57	0.74	-	0.81
Urban only			1.09	0.93	2.24	0.62	-	1.10
Georgia								0.72
Rural only	Wilkins, 1972	5-1/2	0.91	0.45	1.39	0.39	-	0.65
Urban only								1.18
							Chinese	
Alameda County, CA (98% urban)	Linden, 1972	10	2.53	1.05	3.59	0.048	5.98	1.84
Calhoun county, MI (65% urban)	Seidman and Kreiman, 1972	21	-	-	-	-	-	1.90
Connecticut	Foote, 1967	36	-	-	-	-	-	2.65
							Eskimo	
Washington/ Alaska	Farwell, 1972	12	5.74		8.42		0.30	5.13
Total U.S.	Biometry Branch, 1971	1	2.7	1.4	4.8	1.4	-	2.2

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TABLE 2

Primary liver cancer age specific rates from available United States state cancer registers - comparison of the Southeast with other areas of the United States

Registry	Age group	Age specific rates, all races, both sexes (cs/10^5/yr.)
Alabama, 1959-1972	37-50	0.83
Georgia, 1967-1972	36-45	0.25
	46-55	0.67
Connecticut, 1935-1970	35-39	1.0
	40-44	1.3
	45-49	3.6
	50-54	5.8
Alameda County, CA, 1960-1969	35-39	0.2
	40-44	0.8
	45-49	2.1
	50-54	3.4
Total U.S., 1969 Based on SMSA* data	35-44	0.9
	45-54	2.4

*SMSA = Standard Metropolitan Statistical Area.

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NON - EXTRUSION TEXTURIZING OF SOY MEAL

M. M. Sterner, M. H. Sterner and G. Zeidler*

The term textured vegetable protein (TVP) applies to a variety of new foods produced by spinning of protein monofilaments or extrusion, or extrusion puffing of a proteinaceous raw material. Nearly any of the commonly available vegetable or other protein sources can be used, and the products are becoming widely used. The United States Department of Agriculture estimates that 40 million pounds of textured vegetable protein are used yearly in that country's school lunch program. U. S. manufacturers are making volume sales of TVP as meat extenders in products such as hamburgers and Mexican-style foods sold by franchised "fast food" retailers.

Textured Vegetable Proteins in Developing Countries

The U. S. acceptance of textured vegetable protein has prompted other countries to become interested in this low-cost answer to the demand for more protein foods. The United Kingdom, Sweden, Israel, South Africa, Japan, Mexico, and India are among the countries that have joined the United States in commercial production. However, two major deterrents have stood in the way of textured vegetable protein production in the developing countries. These are: a) the high capitalization costs of imported equipment and b) the high degree of operator sophistication required to successfully texture proteins in the traditional basic extrusion cooker.

In an attempt to circumvent these difficulties and find a way to make low-cost textured vegetable protein foods at the village level, Meals for Millions has investigated a simple apparatus embodying the elements required for texturizing proteins but heretofore not used for this purpose.

Non-Extrusion Cooked Textured Vegetable Proteins

Prior research by Touba disclosed that "masticating (the protein) mix at temperatures substantially above the boiling point of water" is unnecessary. He demonstrated that texturing of protein material will occur when the mix is compressed between two hot plates and released (1). His patent titled "Texturing of Protein", assigned to General Mills, requires the protein content to be at least fifty per cent or more, and that of moisture to be between fifteen and thirty-five per cent.

Cognizant of these findings, Meals for Millions (MFM) in February 1973 began testing the practicability of texturing protein in a simple apparatus used for many years in Korea. There, they make a saucer-shaped, puffed snack food from rice-like pellets of sweet potatoes. Such an apparatus can be purchased in Seoul for approximately $42.00. This apparatus is pushed to a street location on a small cart. A base plate and flanged plug type lid are heated by a coal briquet fire. For MFM's purposes, an electrical heating element with intervening rheostat was attached to the base plate and lid. Instrumentation was added to measure pressure and temperature simultaneously.

Operation of a Simple Texturizer

Texturing of protein material is accomplished by placing a predetermined quantity of a mixture of meal and water into the base plate, then placing the plug type lid in proper alignment and applying the desired pressure to the mix by means of a lever connected to a plunger.

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The level is held down for the desired duration at the desired pressure. The lever is then released instantaneously and the superheated water within the protein mix explodes in vapor and puffs the product, resulting in a fibrous structure.

A Unique MFM Breakthrough: Texturizing Full Fat Soya Flour

It is commonly accepted that the limit of 1 per cent fat prevails in a normal texturing process, but MFM found that full fat soy flour provides a textured product containing 36.7 per cent protein, 20.3 per cent fat, and 30.4 per cent carbohydrates. To achieve and maintain the shape of the product using the village texturizer it was necessary to increase moisture and decrease the temperature.

Short and Long Range Results

The results of MFM's work indicates: a) the possibility of texturing protein in a shape such as a patty or salisbury steak by modifying an existant piece of machinery now in use in order to make possible the production of low-cost textured vegetable protein foods at a village level; b) gives researchers a tool for investigation in their search for answers as to what really is happening in the "black box" of an extrusion cooker; c) the prospect of using full fat soybean flour increases the usefulness of the apparatus for the purposes for which the modifications have been intended; d) makes it possible for developing countries to produce textured vegetable protein without heavy capitalization; the modified apparatus can be manufactured at the local level for about $50.00; e) operation of the texturizer requires a minimal degree of operator sophistication; f) the technology employed in modifying the texturizer is readily transferable to a wide variety of developing countries - a program already in process at Meals for Millions.

Meals for Millions believes that textured vegetable protein production in developing countries is a practical process. As a non-profit organization endeavoring to help solve the world food problem through self-help programs, MFM will provide basic drawings of the village texturizer to anyone wishing them.

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PROCESSED PROTEIN FOODS AND LYSINOALANINE*

Many foods are exposed to alkaline conditions during cooking or processing. Some well-known examples include the treatment of corn with lime in several Latin American countries, and the high pH of cooked egg which results from the loss of carbon dioxide during cooking. Neither of these products has been considered to have any detrimental nutritional or toxicological effect. However, about a decade ago (1) it was discovered that treatment of proteins above pH 10.5 at 25°C or above pH 8 in boiling water leads to the formation of crosslinks in the protein. These crosslinks were characterized after acid hydrolysis of the treated proteins, as a new amino acid trivially named as lysinoalanine (LAL).

Interest in LAL comes from the effect of its formation on the nutritive value of the treated protein and from the reports of renal lesions in animals fed severe alkali-treated proteins. The toxicological manifestations may include nephrocytomegalia, tabular nephrosis, necrosis,

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and regeneration of epithelial cells (2, 3).

Since several types of foods and food processes involve exposure of proteins to alkali there has been some cause for concern about LAL, particularly in the case of protein isolates which are manufactured by alkaline extraction processes. A recent paper (4) summarized the many other changes which proteins can undergo upon severe alkali-treatment, including loss of cystine, arginine, threonine, serine, isoleucine and lysine. In addition to LAL, other unusual amino acids, including alloisoleucine, ornithine, and lanthionine, and racemization of lysine also occurs during this treatment. The presence of such crosslinks as LAL and lanthionine in a protein would help to explain reduced digestibility and amino acid availability, but only the formation of LAL could be construed as presenting a hazard to the general public.

Most of the changes listed above require fairly severe conditions of temperature and pH not likely to be encountered by proteins destined for human consumption. In a study on a commercial type of spun soy isolate, the investigators were unable to detect nephrocytomegalia (5) leaving the question open of whether LAL could be found in any significant quantity in this type of product.

As a result of the apparently conflicting information on the toxicological significance of LAL formation in proteins, it seemed important to Sternberg and co-workers (6) to determine the extent of LAL distribution in commercial food products. To this end they developed a simple procedure utilizing thin-layer chromatography for analysis of LAL (7) which enabled the investigation of a large number of food products, cooked under a variety of conditions. The unexpected results of this study are that LAL is widely distributed in cooked foods, commercial food preparations and food ingredients. Concentrations found varied widely, from as low as 50 ppm of the protein in a boiled frankfurter to as high as 50,000 ppm in a commercial whipping agent.

Among foods which are particularly susceptible to LAL formation are egg white and milk or milk proteins. Ege white varied from a level of zero for uncooked to 350 ppm for 10 minutes pan-fried, to 1820 ppm for a commercial dried sample. A variety of infant milk formulas and/or condensed or evaporated milk contained from 150 to 860 ppm. In contrast, the range for 45 commercial samples of soy protein isolate, consisting of different batches from two manufacturers was 0 to 370 ppm. Experiments with purified proteins, including ovalbumin, casein, lysozyme, bovine serum albumin, and soy globulin confirmed that alkaline conditions were not a sine qua non condition for LAL formation, if the foods were also exposed to temperature of 100°C or greater. At these normal cooking temperatures, some proteins form LAL even under mildly acidic conditions.

Sternberg, et. al. (6) conclude their paper by observing " ... our finding of LAL in the structure of heated proteins not necessarily subjected to alkali treatment may be a factor to be considered in explaining reduction of nutritional value by heating. The formation of LAL in proteins by heating at pH values considerably lower than those obtained during alkali treatment ... suggests the ubiquity of LAL in cooked foods and that humans have long been exposed to proteins containing LAL".

What, if any, toxicological significance is there to these findings? The resolution of the problem may be found in the work of de Groot and co-workers (3). Since, as mentioned earlier, feeding a commercial soy isolate containing lysinoalanine did not result in nephrocytomegalia (5), the hypothesis was tested that lysinoalanine is not released from the treated protein by the digestive process. The hypothesis was confirmed by the observation (3) that synthetic LAL or acid-hydrolyzed alkali-treated protein caused the kidney lesions, while the intact protein was ineffectual. Since Provansal, et. al. indicate that Pronase, a potent mixture of microbial proteases, does cause some release of LAL, we cannot exclude the possibility that drastically treated proteins may be nephro-toxic. Apparently, however, the wide distribution of LAL among cooked foods would tend to indicate that this is neither a novel nor serious problem. In addition, de Groot has shown that the rat kidney lesions may be peculiar to that

species, since several other species of animals, including the dog, mouse, hamster, Japanese quail and monkey, do not develop such lesions even with free LAL. Although it cannot be absolutely excluded, it appears only a remote possibility that LAL is a problem for man.

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PROJECT POSHAK - INTEGRATED HEALTH AND NUTRITION LARGE-SCALE STUDY FOR RURAL PRESCHOOL CHILDREN, CENTRAL INDIA

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Project Poshak, an integrated project of nutrition and health services for pregnant and lactating mothers, and for children up to three years of age, was conducted in Madhya Pradesh (Central India) between 1971 and 1975. The operational feasibility, the efficiency of the delivery system, the nutritional impact and the cost-effectiveness of a "take-home" food-delivery system, supported by child-care education and basic medical/health services, were assessed during the course of a three-phase study which involved approximately 13,000 children and 5,000 women in 514 villages in 12 districts. Maximum emphasis was laid on utilizing the existing government rural health network of the country.

The project was administered by CARE and was sponsored by the Government of India and the Government of Madhya Pradesh. Financial assistance was provided by the Government of India, USAID, and UNICEF. An entirely Indian multidisciplinary team of nutritionists, public health personnel, statisticians, pediatricians, sociologists, and logistics experts planned, designed, trained, monitored, and evaluated the project. The day-to-day implementation of the project was the responsibility of 88 medical officers and 400 paramedical government staff who were deputed on a part-time basis by the Health Department of Madhya Pradesh. Thus, a large complement of the total health staff of the State were trained to expand the project into a program.

Madhya Pradesh offered a particular challenge for the development of health and nutrition delivery systems, in view of its immense logistical and operational problems. In area, it is larger than Japan or Malaysia, has a population of 42 million, 25 per cent of whom are tribal. More than 70 per cent of the target population in the study had an annual per capita income of $28 to $36, well below the poverty line. Approximately three-quarters of the children enrolled in the study were in the second or third degree of malnutrition (Gómez classification). More than 90 per cent of the target population was illiterate.

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The package of services consisted of a food supplement, health service and maternal and child care education. A 700-g ration (100 g/day) per child and an 1,400-g ration (200 g/day) per mother of ICSM, a precooked corn-soya-milk powder (sweetened and flavored) were distributed weekly from the PHC. The educational input consisted of actual demonstrations, flip charts, growth charts, films and slide shows, and was dispensed fortnightly through interpersonal contacts at the village. Health and medical services consisted of fortnightly village clinics, including immunizations, deworming, and curative services. Paramedical staff visited the homes once a fortnight to follow up education and medical advice and to emphasize the usefulness of the food supplement for the beneficiary.

The project began with an Exploratory phase (intervention period of 7 months), which provided the take-off point for two subsequent phases of the Project: the Extensive (intervention period of a year), which was conducted over a large geographic area under minimal conditions of supervision; and the Intensive (intervention period of 16 months), which consisted of several in-depth studies. These included the production of weaning foods from locally available foods; the effect of pricing a food supplement; the utilization of the village school as an alternative distribution center to the health center; the impact of single and combined health and nutrition inputs on nutritional status; the cost-effectiveness of "on-site" feeding versus "take-home" feeding approaches; and, lastly, the educational impact of nutritional rehabilitation on severe PCM cases in the child's home itself.

The major conclusions and recommendations of the study were as follows:

1. It was operationally feasible to integrate MCH nutritional programs using the take-home food approach with existing rural health program of the country. The cost of delivering the package of services was $28 per child per annum.

2. The take-home food approach was powerful educational tool for largely illiterate communities.

3. There was a tremendously enhanced utilization (33 to 41 per cent) of the health centers. Acceptance of MCH services, immunizations, and even birth-control methods showed very encouraging upward trends in those communities that received the services.

4. The nutritional status of the intervened groups of children demonstrated that improved nutritional status against their age matched nonintervened counterparts, as evidenced by a better weight-for-age status, fewer episodes of illness, and enhanced knowledge gains in child-care practices among the mothers.

5. It was feasible to attach small food-mix units to the health centers to produce weaning and toddler foods from locally available grains, pulses, oilseeds and jaggery. These units could form an integral part of the rural health center complex.

6. The package of health and nutrition services gave the best nutritional impact with respect to the young child. However, results almost as good were achievable with a high level of health care, supported by a relatively low level of a good supplement, which indicated that far more cost-effective, manageable, and wide-coverage programs must be explored, using optimal mixes of health and nutritional delivery for maximal impact.

7. Approximately half the take-home ration reached the toddler. The food was shared by siblings up to the age of six years. Inclusion of this older age group (4-6 years) would greatly minimize the diversion of food. Approximately 1/10 of the take-home food ration reached the pregnant woman and approximately 1/4 reached the lactating mother. This points to the need for developing special mother foods, which would be acceptable both culturally and psychologically.

8. In countries with slender resources, there is a definite need to experiment with feeding approaches that relate to the seasonal need of the populations. Food is most required during the lean, drough-prone summer months.

9. Take-home food approaches are as efficient as "fed-on-site" systems for 2/3 the cost; the potential outreach of the former is more than double that of the latter.

10. Reaching the poorest of the poor will always be a problem, as their participation in welfare programs is limited. It may be possible to reach this segment of the population partially through properly organized Food-for-Work schemes that generally attract them. The possibility of using mobile creches to render health and nutrition services for the vulnerable children of these families needs to be explored.

11. Even nominally priced supplements are out of the reach of those below the poverty line.

12. The village school offered a good alternative as a distribution center for the very young child, and should be explored.

13. Child-care education must be imparted to the young mother substitutes (aged 7 to 10 years), who generally care for the very young child when the mother is at work.

Programs based on Project Poshak have already begun in India with the assistance of CARE-India. The ministry of Health, Government of India, has started an experimental program called Suraksha (protection), in which children (0-4 years) of family-planning acceptors in the National Post Partum Family Planning program will receive a package of Poshak-type of service. It is hoped that such an approach will make the best use of scarce resources, will attempt to insure better child health, and will motivate greater numbers from the socio-economically depressed sections to accept birth control methods as a way of life.

The planning and organization of the project, the methods used and the detailed results have been brought together in a two volume publication titled Project Poshak by CARE India, B-28 Greater Kailash I, New Delhi-110048, India in 1975. The project director Dr. Tara Gopaldas is the author of the publication.

Volume One (328 p.) contains: 1. Preamble to Project Poshak, 2. The Exploratory Phase of Project Poshak, 3. The Extensive Phase of Project Poshak, 4. The Intensive Phase of Project Poshak, 5. Efficiency, Cost and Econometric Studies, 6. Programs Based on Project Poshak.

Volume Two (248 p.) contains: 1. Planning Process, 2. Site and Target Selection, 3. The Food Component, 4. Childcare Education Component, 5. The Medical Services Component, 6. Training and Information Dissemination, 7. Program Management, 8. Monitoring and Evaluation Methods, 9. Conclusions and Recommendations.

BOOKS

Action for Children. Towards an Optimum Child Care Package in Africa. (Ideas and proposals based on the proceedings of the Dag Hammarskjöld Seminar held in Addis Ababa, 14-19 May 1973). Edited by Olle Nordberg, Peter Phillips and Göran Sterky. 1975. Published by Dag Hammarskjöld Foundation, Övre Slottsgatan 2, S-75220 Uppsala, Sweden. 238 pages. Price airmail Skr 40/$. Surface mail Skr 30/$.

Despite many efforts in the past and currently undertaken, the minimum health and nutritional needs of the growing child population in many developing countries have not been met even partially. There are several reasons but they are not new. The hostile environment, poverty, lack of adequate water supply and sanitation and insufficient information on the specific cultural and other local factors responsible for the occurrence of diseases and how to apply

preventive measures in the local context are some of these. Nonavailability of trained personnel and inadequate resources are equally important. It is unfortunate that the health and nutrition strategy commonly adopted in these countries are simple copies of the expensive health and medical set up of the developed nations. The hospital oriented, conventional curative medical approach is largely concentrated in urban areas where only a small percentage of the people lives.

This book reviews the problems and their solution, as applied to Africa. It recommends the integrated, multidisciplined coordinated approach to community health and nutrition services closely linked to local needs and values. Such an approach should motivate the community and obtain its local cooperation, support and committment.

More than two thirds of the book is devoted to a background presentation of the magnitude of the socio-economic problems and of the extent and severity of illhealth and malnutrition during early years of life. An attempt is made to relate these to answer the questions why an integrated people oriented approach alone will succed and how the strategy for such an approach should be developed. A brief section titled "Dilemma" gives concrete examples of the deficiencies of the existing urban oriented system and how it breaks down easily under the stress and strain, so common in these situations.

The last section outlines the strategy in the form of a Child Care Package and its delivery. Although the strategy is described as new, the different elements of the package are well established items in one or the other child health care programs in operation all along. This package program is recommended as the most promising approach in the face of severe resource limitation in these countries and the simultaneous application of the different inputs of the packet is believed to provide a multiplier effect on the total health benefits, leading to an upgrading of the quality and quantity of child care available.

Population, Health, Nutrition and Development: Theory and Planning. Hector Correa. 1975. Lexington Books (div. D. C. Health), Lexington, Massachusetts, U.S.A., U.S. $21.50, 219 pp. and index.

The aim of this book is to study the application of the techniques of systems analysis, operations research or mathematical optimization to the planning of human welfare aspects of development. The early chapters briefly present interactions, translated into mathematical terms, between population growth, nutrition, health and socioeconomic development. These chapters also provide a rationale for the use of mathematical techniques for planning in those areas. The remaining chapters provide a collection of operations research models for use in planning population growth, health and nutrition - individually or combined with socioeconomic planning.

NEWS

Texas High Plain Sunflower Tests

With a total global yield of 3.7 million metric tons of sunflowers yearly, sunflower oil is the world's second most plentiful vegetable oil after soybean oil. Sunflower oil is high in polyunsaturates and is in some ways comparable to maize oil. Also, it is quite stable in storage, although not quite as stable as cottonseed oil. Sunflower meal contains about 40 per cent protein, making it a valuable animal feedstuff and worth considering in the future as a protein source for human feeding.

Recent sunflower growing experiments in the high plains of the state of Texas, U.S.A., may have implications for other parts of the world. A report in the May 1975 issue of Agricultural Research, an organ of the U.S. Department of Agriculture, points to ways sunflowers can be integrated into multiple cropping systems and to irrigation methods for increasing yields.

In one of the experiments, scientists at the

USDA's Southwestern Great Plains Research Center in Bushland, Texas, took advantage of the relatively short - about 100 days - sunflower growing season. This meant a likely potential for producing good yields following irrigated wheat in a double cropping system. The investigators studied the growth of sunflowers following wheat using two tillage treatments - clean tillage and no tillage.

Sunflower plants emerged within ten days and competed well despite a large amount of volunteer wheat from the preceding wheat crop due to hail damage estimated at 20 per cent. Yields of sunflower seed from one of the cultivars used were 1758 kg/ha in the clean-till plot and 1960 kg/ha in the no-till plot.

In tests under a variety of irrigation conditions, the scientists noted that seed yield generally increased with increased number of irrigations. A single irrigation at flowering resulted in 277 kg/ha more than those irrigated only for emergence, based on averages for five sunflower cultivars tested. Three irrigations per growing season increased yields by an average 615 kg/ha over seeds that received emergence irrigation only.

In a dry land management experiment, yield variations occurred between sunflowers grown on plots established on summer-fallowed land and those grown on a conservation bench. Yields on the summer-fallowed plot ranged from from a low of 762 kg/ha to a high of 1131 kg/ha. Yields on the conservation bench ranged from 392 to 1142 kg/ha.

The wide variation in yields among the cultivars tested was attributed mainly to differences in plant populations and irregular seed emergence. The latter caused problems at harvest and resulted in lower yields because seeds were lost from older plants while younger plants were still immature. Since the price per unit weight of sunflower seeds was, at the time of the experiments, three times that of sorghum, the high yield of 1142 kg/ha compared favorably with grain sorghum yields of 2016 kg/ha on similar plots.

The scientists found that the sunflowers suffered very little damage in a hailstorm that stripped the leaves on grain sorghum and destroyed soybeans on nearby plots.

International Food Policy Research Institute

The Ford and Rockefeller Foundations recently joined with the International Development Research Centre of Canada to support the establishment of an International Food Policy Research Institute. Located in Washington, D. C., the new Institute is directed by Dale Hathaway and will have a staff of a dozen scientists drawn from both developed and developing countries and an initial budget of $1 million/biennium. Its mission is to provide information and analyses concerning the world food situation and to conduct research on international policy aspects of food production, distribution, and trade. It will cooperate with FAO and other international groups in identifying gaps and opportunities for expanding world food production, and is expected to recommend actions that governments and international organizations might take to improve production, trade, and distribution of foods.

(Notes from Washington United States Department of Agriculture, Agricultural Research Service, International Programs Division, Volume 76, Number 1, March 1976).

$19 million World Bank loan to Brazil for nutrition project

The World Bank is loaning $19 million to Brazil towards financing a $72 million project that will strengthen the nation's ability to upgrade the nutritional status of its population. The project is actually the cornerstone of a more massive structure - a planned $1.3 thousand million comprehensive national nutrition program. The World Bank's commitment is its first in the field of nutrition, while Brazil's is the largest ever undertaken by a country to combat malnutrition.

(continued on back cover)

PROTEIN-CALORIE ADVISORY GROUP

The Protein-Calorie Advisory Group of the United Nations System (PAG) is an interdisciplinary committee of internationally-recognized experts who advise the United Nations and its agencies on technical, economic, educational, social and other related aspects of global malnutrition problems and the broad programs and new areas of activity needed for combating them. Since its inception in 1955, the PAG has emphasized protein-calorie malnutrition as a primary and continuing threat to the health and survival of infants and young children in the developing countries and has played an active role in promoting the development of novel and locally-available protein resources for the developing world. The PAG also reacts to socioeconomic considerations, trends in world food supply and consumption and the need for governmental initiatives and priorities in dealing with these problems.

The PAG is sponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Bank for Reconstruction and Development (IBRD), and the United Nations.

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The purpose of the PAG Bulletin is to promote the exchange of information on the world malnutrition problem among all those who are motivated to work towards its solution. Published quarterly in English, French, and Spanish editions, it is sent gratis to individuals, institutions, and commercial organizations with an active interest in scientific, technological, economic or social aspects of protein-calorie malnutrition on a worldwide basis.

The PAG Bulletin can succeed in its mission only insofar as it can comprehensively and objectively communicate with its readership. Readers are invited to comment in writing on what they read in the Bulletin. In addition, the PAG Secretariat welcomes suggestions for broadening and deepening the scope of the Bulletin, thereby increasing its usefulness.

Mailing list
Every effort will be made to honor requests to be added to the PAG Bulletin mailing list. In the interests of economy, however, copies should be shared by colleagues or borrowed from libraries wherever possible. Address requests to:

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(continued from page 40)

Brazil is the biggest and most populous country in Latin America. As in many other developing countries, malnutrition in Brazil is widespread. It is particularly acute in parts of the Northeast where infant mortality - largely related to malnutrition - is reportedly highest in Latin America.

The four-year program as well as the project in cooperation with the World Bank are based on the assumption that malnutrition is both a consequence of underdevelopment and contributive factor, requiring integrated responses to alleviate poverty and to broaden economic opportunities. Another assumption is that even in the absence of complete data on malnutrition and of methods for quantifying the problem, a well-directed investment in nutritional improvement can lessen morbidity and mortality, contribute to increased productivity, assist in achieving educational objectives and serve as a means for income redistribution.

Besides taking measures to meet current needs the program will lay a foundation for longer-term measures through rationalizing food production, marketing and distribution; support of small agricultural and industrial producers; and improving the capabilities of institutions responsible for formulating national nutritional programs.

The World Bank-financed project adds an important set of policy, analytic and institutional dimensions to the overall program. The project will help to establish the information base needed for formulating longer-term policies and programs to combat malnutrition. An innovative aspect of the project is the creation of a mechanism in the Ministry of Agriculture to analyze the nutritional consequences of price policies and other agricultural programs.

As part of a test of four alternative delivery systems to provide better nutrition to the people, the project will introduce a novel concept for nutrition interventions - the combined use of agricultural and social extension to improve simultaneously the nutrition and productivity of low-income farm families. The project will also introduce industrial incentives leading to the development and production of low-cost nutritious food and food fortificants produced locally. In addition, the project will concentrate on improving Brazil's capability to identify and carry out major long-term nutrition programs, particularly through a newly established National Food and Nutrition Institute.

The World Bank's loan to Brazil is a response to the World Food Conference's resolution calling on international agencies to assist countries in preparing national food and nutrition policies and programs.

ERRATUM

PAG Bulletin, Vol. V, No. 3, 1975, page 26, column two, lines 8 and 9 should read:

"It would appear that the recommended adult level of nucleic acid intake for SCP supplementation ..."

VOLUME VI, NUMBER 3, SEPTEMBER 1976

PAG BULLETIN

Protein-Calorie Advisory Group
of the United Nations System

Food and Agriculture Organization of the United Nations

World Health Organization

United Nations Children's Fund

International Bank for Reconstruction and Development

United Nations

PAG Bulletin

Volume VI, Number 3, September 1976

COVER:

SCP (yeast) "Photographed" at 4600 times magnification, using the electron scanning microscope. Note the cell at far left edge just beginning to "split" or double (Photo taken and kindly supplied by Provesta Corporation and its parent company Phillips Petroleum Company).

IN THIS ISSUE....

PAG Bulletin, Vol. VI, No. 3, September 1976. *PAG Bulletin* is published quarterly in English, French and Spanish editions by the Protein-Calorie Advisory Group of the United Nations System, Room 606, 866 United Nations Plaza, New York, N.Y. 10017, U.S.A. Controlled circulation postage paid at New York, N.Y., U.S.A.

PAG BULLETIN

VOLUME VI, NUMBER 3
SEPTEMBER 1976

SYMPOSIUM ON HYDROCARBON-GROWN SINGLE CELL PROTEIN PRODUCTS FOR ANIMAL FEEDING: AN INTRODUCTION

The Protein-Calorie Advisory Group (PAG) of the United Nations System has played an active role in promoting the development of various novel protein food sources, and, as a part of this activity, has been monitoring research and development on single cell protein (SCP) products through a series of Working Group meetings. So far, five such meetings have been held, and they have reviewed all available data on the production and use of SCP for both animal and human consumption. Based on these discussions, the PAG has issued several recommendations, statements and guidelines on different aspects of SCP production, evaluation, safety, testing, nutritive value, etc. These PAG documents, which are widely accepted, have also helped to focus attention on problems or potential problems, and to identify additional needs for study and research.

Many commercial enterprises have launched successful programs leading to production of SCP products for animal feeding. Seen as a long-term possibility a few years ago, SCP production has now become an industrial reality. Since SCP production is a rapidly moving field, the PAG recommended early in 1975 that the SCP Working Group meet in the near future to review the progress in this area. It was also recommended that PAG hold a symposium on SCP for animal feeding, so that the new information and experience would be helpful to update various PAG recommendations on SCP, particularly those relating to quality and safety evaluation of novel protein sources for animal feeds.

With these objectives, the PAG sponsored an international symposium in Brussels, Belgium, on 29 and 30 March 1976. The three sessions held were devoted to discussions on technological developments and experience with SCP grown on hydrocarbons, toxicological aspects, and regulatory aspects, respectively. The new information introduced at this symposium, and the additional knowledge gained as a result of the discussions, greatly helped the sixth PAG Working Group meeting, which immediately followed the symposium to critically evaluate current trends.

The report of the sixth PAG Working Group meeting and the summary of the symposium have been published in the PAG Bulletin Vol. VI, No. 2, 1976. In this Bulletin, a few technical papers discussed at the symposium are presented. The remaining papers will appear in future issues of the PAG Bulletin. The Proceedings of the Symposium will be published separately in the near future.

P.S. Venkatachalam
Deputy Director
PAG Secretariat

POTENTIAL IMPORTANCE OF NEW PROTEIN SOURCE - KEY NOTE ADDRESS

Lord Ritchie-Calder*

My introduction to single cell protein was while the bombs were falling around us in the London blitz. Night after night, and all night, people took refuge in public air raid shelters improvised in the London underground railway or in underground warehouses. The assumption was that people would go down into the shelters when the sirens sounded the alert. The raid would pass and the all clear would sound and they would go home and cook their own meals. It did not work like that. The raids were intermittent and the alerts continued all night. Families moved into the shelters at dusk and emerged at dawn. Breadwinners joined them without any supper and left without breakfast. Something had to be done about feeding them, but conditions in the shelters were scarcely conducive to hygienic cooking.

Chaim Weizmann, in his capacity as an industrial chemist, provided an answer. With his colleague, Ernst Bergmann, he had been working on yeast. He had followed up the work of Richard Willstater and his pupils, who had shown the powerful protein splitting enzymatic action of the yeast cell. It could be shown that the enzymes were released in a most active state if the yeast were plasmolysed and made to give up the liquid content of the cells. Plasmolysed yeast could break down otherwise indigestible vegetable protein into amino acids and, more than that, the protein of the yeast cell itself provided amino acids lacking in the vegetable matter.

The first time I sampled Weizmann's yeast was at a dinner party at which he himself was present, in the winter of 1940, when the bombing of London was at its worst. It was served as a luscious borscht. I was not interested in either the scientific or the Galloping Gourmet aspect, but whether it could be used to feed the hungry people in the shelters. It could. With the help of a public-spirited commercial firm, Blitz Broth was produced. The chief scientist of the Ministry of Food, Sir Jack Drummond, cooperated in establishing the recipe, which embodied Weizmann's meat substitute without recourse to rationed meat and using off-ration vegetables. The broth was prepared in commercial kitchens and shipped in thermal containers to the shelters, so there were no hygienic hazards. The nutritionists reckoned that, with a hunk of brown bread, a large bowl of Blitz Broth could keep a manual worker going for twelve hours. It was well-received by the shelter-dwellers; and among the mixed religious communities of London's East End it could be guaranteed taboo-proof.

The material used was peanut cake. At that time, Britain was importing peanuts, the oil of which was used for rationed margarine and rationed soap. The residue, which would have been used for cattle-feed, was converted into a palatable and digestible protein for human consumption.

As a historic footnote: Blitz Broth, in self-heating cans, provided the iron ration for British paratroopers dropped into Normandy on D-Day.

Here was the upgrading into a satisfactory, and desperately needed, meat substitute of a waste product - "waste", that is, in terms of the primary requirement, in this case the oil for margarine and soap.

What is waste, in view of the limitations of the Earth's resources and of the aggravation of pollution?

I will define waste. Waste is industrial myopia. It is a short-sighted concentration on a prescribed product, which discards, as superfluous, materials irrelevant to that

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*Member, House of Lords, Westminster, London, England.

outcome. The discards may contain a wealth of materials which are recuperable or convertible.

At the same time that we were using Blitz Broth, Britain was putting another micro-organism to work - Penicillium notatum. Florey and Chain at Oxford University were producing the first penicillin as we know it. Their equipment for manufacture consisted of a set of milk churns, a dog-bath, an air raid precautions' stirrup pump and a milk-cooler borrowed from a dairy. The molds were grown in milk bottles and bed-pans. The nutrients were shipped by road to Oxford from a brewery in the East End in London, which was under bombardment. Britain, going it alone, and with all its engineering capacity devoted to armaments, could not divert scarce resources to creating a mass production plant. A mission was sent to the United States to enlist the cooperation of American technology. The requirement was deep-culture, and the question was how to obtain an abundant supply of nutrients. Dr. R.D. Coghill, Head of the Fermentation Division of the United States Department of Agriculture at Peoria, Illinois, suggested the use of corn-steep liquor, a waste product which at that time was a costly embarrassment because it was so rich in nutrients and consequent eutrophication that it could not be discharged as sewage. Thus penicillin, which in the following decade was to save more lives than had been lost in all the wars of all human history, was produced from "waste".

My function, if I may arrogate one, is concerned with the social effects of science and technology. In that capacity, my interest in SCP is two-fold: first, and most, I see SCP as an augmentation of the world food supply; and second, I view research on SCP as a sensible, indeed imaginative, way of finding alternative uses for materials which we otherwise squander as waste.

Chaim Weizmann, who once said, "Bugs are cheaper than B.Sc's," maintained that the use of a biological method for the predigestion of waste protein instead of a chemical method has the advantage that no decomposition of even the most sensitive amino acids takes place, and that the stereochemical configuration of the natural amino acids is maintained. Furthermore, he claimed, a biological method, proteolysis, is more likely to keep intact the still unknown nutritional factors which are contained in the protein.

This latter remark is reassuring to me because, over forty years ago, my friend, Sir Frederick Gowland Hopkins, that trail-breaker in the science of nutrition and Nobel Laureate for his work on vitamins, said to me: "Vitamins are units of ignorance and not of knowledge. Every new food factor which we discover should remind us of the food factors we have not discovered."

He had the proper humility of a great scientist, reacting against what he called "the lusty self-confidence" of the nineteenth-century chemists. Secure in the paramountcy of Liebig, they nearly exterminated the British aristocracy. Because Liebig had been taken too literally, baby foods became fashionable. They offered liberation from the dependence on the plebeian wet-nurses, who had suckled the scions of the British artistocracy. There were Balanced Bottles for Bouncing Babies, but with consequences which the chemists had not foreseen, because they had ignored Claude Bernard's injunction, "True science teaches us to doubt and in ignorance to refrain." Bottle-fed babies suffered and died from deficiency diseases, notably infantile scurvy (Barlow's Disease). There was a conspicuously high mortality rate among the children of the gentry.

The term "single cell protein" has seemed to me, as a science writer, an unduly restrictive description, and I was grateful when PAG Statement No. 4 offered what we call in the House of Lords, in relation to a bill, "the extended title." That is, "SCP has become the generic term for crude or refined sources of protein, the origin of which is unicellular, or simple multicellular, organisms, i.e., bacteria, yeasts, fungi, algae and perhaps protozoa and even

bacteriophages."

That is scarcely a lyric to be set to pop music, but it does give imaginative scope, away from the confines of the laboratory, for thinking about the husbandry of micro-organisms as we think of the farming of macroorganisms, the animals and plants. Not that the domestication of micro-organisms is something new. We remember that Saccharomyces were recognized and cultured as brewer's yeast in Mesopotamia by 6000 B.C.; that yeasts, used for making beer and bread, were found in the Egyptian tombs of 2000 B.C.; that the Eber Papyrus shows that yeast was being used in prescriptions in the 16th century B.C.; and that the school of Hippocrates in Cos prescribed yeast for certain debilities (vitamin B therapy in the 5th century B.C.!). In those days of Women's Lib., I should point out that the terms "Malsters" and "Brewsters" are feminine gender, that the earliest mycologists were probably women, and that the housewife who made the beer also leavened the bread.

Like the man who was surprised to find that he had been speaking prose all his life, human beings without realizing it have been eating SCP for millenia. Any fermented food or drink contains cellular organisms. By a long process of trial and error and acquired habits, people of all cultures have assimilated SCP in their diets, but rarely do they identify the organisms or the substrates with the end-product that they find appetizing. To most people, fungi mean decay; bacteria mean the pathogens of disease; and algae, the green scum on duck ponds or the slime of the sewage outfalls. This may be a public relations problem which could be handled, but there is another and more important problem which has been recognized by the PAG. In the commendable concentration on SCP as a hopeful response to the world food crisis, the millenia of trial and error are being concentrated into years of research and development. As the PAG guidelines have stressed, this calls for utmost caution. I repeat Claude Bernard's injunction to his self-confident fellow scientists over a century ago. "True science teaches us to doubt, and in ignorance to refrain." He did not mean that scepticism should hinder progress, but that scientists should feel their way, as though entering a minefield with a mine detector, to make every forward step safe. This is the embodied wisdom of the PAG guidelines, which I heard expressed by Professor Nevin Scrimshaw in his Underwood-Prescott Lecture at the Massachusetts Institute of Technology. He said, "When considering the use for human consumption of either novel proteins or of protein from a conventional source that has been processed in a new or novel manner, the need for the most careful consideration of safety becomes absolutely essential. Extensive prior testing in experimental animals must be followed where appropriate by cautious systematic tolerance studies in human subjects. In many cases, studies on experimental animals alone are not sufficient to eliminate all possibilities of adverse reactions in man, because symptoms which appear in human trials may not arise in experimental animal tests."

There seems general agreement that the preferred SCP products now in commercial-scale production are nutritionally beneficial and harmless to animals, but it has also been stressed that the criteria for acceptability of those novel materials must include concern over residues of components of the feedstuffs which might find their way into human foodstuffs, such as milk, meat (including organs) or eggs, which are derived from the animals.

It is sincerely to be hoped that the wisdom of the PAG guidelines will find its way into the protocols of the commercial producers and the ordinances of government. They are reminders that a short cut is often the long way round. Any misadventure which alarms the public could set back for years the much needed development of SCP.

The more innovative the food product, the more difficult it is to educate the consumer and, as a corollary, the more reluctant are commercial producers to face the capital

risks and long development lead-times and costs of promotion. Moreover, the only way in which hungry people in the developing countries will get the benefits will be as the spin-off from more lucrative marketing.

It is not just a question of return on investment. A foodstuff implicitly labelled "For the Poor" will be as resented as gruel in a Dickensian workhouse (even though Oliver Twist wanted a second helping). Samuel Johnson once said, "Oats are what Englishmen feed to horses and Scotsmen feed to themselves." However, when I was a boy in Scotland, Indian Corn was something we fed to chickens and would never have dreamed of eating ourselves. Now it is acceptable everywhere, dressed up as packaged cereal. Similarly, any SCP product will have to have the endorsement of the better-off countries. It will not be acceptable, however wholesome, as an animal feed being offered for human consumption in developing countries.

When I first heard of nonphotosynthetic single cell protein, I wrote enthusiastically about food factories being attached to every refinery, and envisioned the microorganisms consuming the waste. I thought of the negative cost substrate - something the oil companies would be glad to get rid of. "Food from Pollution," I wrote. Of course, I had the world's hungry in mind, but I received indignant letters protesting the idea of feeding underprivileged people on "oil bugs" and asking about "hydrocarbon carcinogens?" It was a reminder of that social lead-time which is just as important as the research and development lead-time. I, too, had to be educated.

The interest in SCP is very stimulating. Here is a meritorious breakthrough. It is probably one of the points of departure in human history. In the nineteenth century, the New World of the Western Hemisphere was invoked to feed the multiplying millions of industrial Europe. The Great Prairies were opened up and the food supplies went back to Europe. Now we are invoking the world of five hundred million years ago, when the Palaeozoid seas were producing the organic life which became the deposits of oil-hydrocarbon.

Oil, which was predominately the determinant of the Green Revolution has, in our discussions here, become the substrate of an entirely different kind of revolution. Let us consider it. The Green Revolution was the sublimation of conventional agriculture. With the help of the plant geneticist we have produced a grain revolution which did not radically change for a half million years. The grains which were evolved in the process of history and were modified by conventional plant breeders, were taken over by the systematists, who, with genetical certainty, could arrange higher yields from existing acreages with certain limitations. There is no such thing as a free breakfast. To fulfil the geneticists' promises, Nature demanded lots of water (i.e., diesel-pumped irrigation), lots of fertilizer (i.e., from oil sources), and lots of pesticides (i.e., from oil sources). The economics of the Green Revolution demanded broad acres, oil-driven tractors, and, in the pay-off to the hungry, the transport to get it into their bellies. With the oil crisis, the potential beneficiaries of the Green Revolution became the hostages of the oil conflicts of the advanced economies. Oil, that legacy of five hundred million years, has become, in a life-or-death sense, the determining factor for thousands of millions of people. At a critical moment in the history of the survival of homo sapiens, we can, with misgivings, accept the conventional role of oil or break through to an entirely novel role for oil - as a direct source of food.

We do not have to be simple-minded and underestimate the research, development and industrial difficulties of such a breakthrough, but we can be seized of that climacteric significance of being able to collect on food stamps issued five hundred million years ago.

In actuarial terms, it is tolerable. If we calculate, as I do, on a likely population of 9,000,000,000 in fifty years time, and

if we calculate that two tons of petroleum can produce by accepted methods, one ton of pure protein, then we could supply all those people with fifty grams of protein from an annual one hundred sixty million tons of oil, which is about ten per cent of the present production.

This, of course, is a grotesque caricature of the food situation, unless we think of everybody living on the equivalent of yeast pills and ignoring the physiology of the gastric system. It can, at least, reassure us that SCP from non-photosynthetic single cell protein can, in an actuarial sense, supplement the diet from conventional sources.

As a concerned bystander, I take note of the encouraging advances. I note the yeast protein produced by the British Petroleum Company in terms of its Candida yeast, grown on gas oil at Cape Lavéra, and (much nearer home to me in Edinburgh) the use at Grangemouth of Candida in a sterile process using highly purified alkane fraction. I have learned of the ICI work on methanol and I have been impressed with the reassuring data (including acute, subacute, and chronic toxicity studies with rats; and carcinogenicity and multiple generation studies with rats and with farm feeding animals), I would, in the vernacular of the British House of Lords, "wish it a fair wind." Similarly, I am impressed by the industrial production of yeast from n-alkanes in the USSR. They have followed through in experimental animals, in agricultural animals, and in human volunteers, with no red alerts. The studies at least affirm the safety of hydrocarbon SCP in animal feeding and, with a favorable presumption, in human feeding.

I have paid attention to the availability of hydrocarbon substrates - the petroleum substrates: crude oil, gas-oil refined fractions, such as n-paraffins, and so on; but I come back to my first concern that we can make food out of manifest waste. I cannot forget this idea, having seen in the Saharan oil fields the pillars of fire by night and by day. These torches, cathedral high, burn off the unwanted gas. A gas torch of that kind is like arson in a food silo. This was methane gas being squandered as a flare. It could be converted to SCP by bacteria.

On the other hand, alcohols - methanol and ethanol - can be derived by catalytic hydration from gaseous hydrocarbons. Methanol is, by a long start, ahead of methane as a material for fermentation. Both ethanol and methanol can be utilized by a wide variety of microorganisms. They can be prepared in food grade states of purity. They are totally water-soluble and they deposit no residues on the cell-mass leaving the fermenter.

Finally, I want to revert to Claude Bernard. Among his many wise aphorisms, he said to his colleagues over a century ago, "When you enter your laboratory, put off your imagination as you take off your overcoat... lest it hamper you and your powers of observation...but put it on again with your overcoat when you leave."

One might say, like Claude Bernard: Let nothing interfere with the objectivity of your researches. Do not be romantic about possibilities or rash in your scientific assumptions. But "put on your imagination with your overcoat" and see the stark realities of a hungry world.

Today, as a minimal estimate, one-tenth of the world's population goes to bed each night having consumed less fuel-foods than their bodies need. When we consider "food necessary for well-being," the situation is worse. The present increase in mouths-to-be-fed (the survival rate, not the birth-rate) is the equivalent of twenty divisions of Martians invading this planet every day without their field rations.

Unless Man vetoes the evolution of his own species in a nuclear holocaust, there is no way of escaping the fact that the present population will double itself in the next twenty-four years - by the turn of the century. Half the population of the developing

countries where the population explosion is worst, is under fifteen years of age; if we had the most vigorous and successful birth-control campaign in all those countries and if, in marrying or mating, those present teenagers were to respond to that campaign, the world population twenty-four years from now could not be less than twice our present number. It is a biologically committed figure.

Human ingenuity must find the answer in scientific research. We must convince people of the practicality of what is being done and discussed in the area of single cell protein research. I would end with another injunction, this time from my old friend, the late Lord Boyd-Orr, who was the first Director General of FAO. He said, "Take food out of politics and take politics out of food."

EXPERIENCE WITH SCP IN ANIMAL FEEDING IN EUROPE

Dr. Ir. P. VanderWal

Introduction

Single cell proteins grown on unconventional substrates can be divided into two main categories: (a) SCP grown on wastes: These products help to upgrade the nutritional quality of molasses, straw, citrus, potato wastes, etc. However, problems arise frequently due to the inconsistency of substrates and of processing conditions. Well-controlled processing conditions are essential for obtaining a product with a constant quality. Often the limited quantities of substrates available, and the low concentrate of wastes, are inconsistent with the relatively large investments in equipment required for SCP production on these substrates. Some progress seems to have been made with the following substrates: manure (Harmon, 1972), straw (Bellamy, 1973; Nolan & Shull, 1973), wood (Forss, 1972), carob extract (Imrie Righelato, 1976), waste paper (Brown & Fitzpatrick, 1976), and potato wastes (Skogman, 1976). In most cases, the evaluation of these products for use in animal feed was limited. (b) SCP grown on gas oil, methane, and their derivatives: The availability of large amounts of these substrates in standard quality make it feasible to develop and evaluate large scale SCP production systems. The expected scale of production justifies the large research efforts required in this case.

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Composition of SCP

The composition of SCP varies with the nature of the microorganism, its genetic potential, the nature of substrate, and the type of developing process used. Table 1, shows the comparative composition of yeast (Toprina L and G), bacteria (Pruteen), fungi (fungal protein), soya bean oil meal and fish meal.

The crude protein content of the different SCP products is high and comparable with those of conventional protein. A sizable portion of the crude protein consists of non-protein nitrogenous products such as nucleic acids. The highest percentage of non-protein nitrogen is seen in the bacterial product "Pruteen". The crude fiber content is particularly high in the fungal protein. Toprina L and soybean oil meal have comparatively low fat content, largely due to extraction during processing. The amino acid composition of SCP is presented in Table 2.

Table 1. Composition of single cell proteins

Nutrient	Toprina L[1]	Toprina G[1]	Pruteen[2]	Fungal protein[1]	Soybean oilmeal[3]	Fish meal[3]
Dry matter (%)	94.5	95.5	96.9	86.5	87.5	91.0
Ash (%)	7.5	6.0	11.5	2.0	5.7	15.5
Organic matter (%)	87.0	89.5	85.4	84.5	81.8	75.5
Nitrogen (%)	10.6	9.6	12.5	5.1	7.2	10.6
Crude protein N x 6.25 (%)	66.2	60.0	78.1	31.8	45.0	66.2
Amino acid N x 6.25 (%)	53.2	47.0	57.6	21.6	38.0	54.2
Non-protein N x 6.25 (%)[4]	13.0	13.0	20.5	10.2	7.0	12.0
Crude fat (%)	1.0	9.0	4.9	4.8	1.0	8.1
Crude fiber (%)	--	--	--	28.0	6.0	--
Nitrogen free extract (%)	19.8	20.5	2.4	20.0	29.8	1.2

1. ILOB data
2. ICI data
3. Data of the Central Bureau for Livestock Feeding in the Netherlands
4. Calculated from total N - anhydro amino acid N

In experiments with poultry, pigs, and calves, the availability of lysine and the true digestibility of the separate amino acids in the yeast have been shown to be generally high. In the bacterial protein, the lysine content is somewhat lower: however, the methionine + cystine contents are higher than in the yeast. For lysine, as well as for methionine, the fungal protein shows the lowest amount. When maize provides a large part of the protein in the animal ration, the relatively low tryptophan content of the bacterial protein (Pruteen) limits its use as protein supplement.

Nutritional Value and Safety

The development of an adequate testing scheme to determine the nutritional value and safety of SCP was the primary concern of ILOB (Instituut voor Landbouwkundig Onderzoek van Biochemische Producten) when originally introduced to these products. A thorough investigation was undertaken because of the novelty of SCP, and the variety of microorganisms, substrates and processes. The testing schedules, developed together with ILOB's sister institute CIVO (Central Institute for Nutrition and Food Research), have been applied since 1965, (VanderWal 1972 and VanderWal 1975). The basic principles of the testing schemes have also been incorporated in PAG Guideline No. 15 (1974).

The testing required for evaluation of SCP for use as animal feed is indicated in Table 3.

Table 2. Amino acid contents of single cell proteins in grams per 16 g/N

Amino acid	Toprina L[1]	Toprina G[1]	Pruteen[2]	Fungal protein[1]	Soybean oilmeal[1]	Fish meal[1]	Requirements for	
							Broilers[3]	Pigs (20-35 kg)[4]
Lysine	7.8	7.0	5.5	4.8	6.2	7.4	5.4	4.4
Methionine & cystine	2.5	2.9	3.1	2.5	2.9	3.9	3.7	3.1
Arginine	5.0	4.8	4.7	5.2	7.2	5.5	6.1	1.2
Histidine	2.1	2.0	1.9	2.0	2.5	2.3	2.0	1.1
Isoleucine	5.3	4.5	3.9	4.1	4.9	4.8	3.7	3.1
Leucine	7.8	7.0	6.3	6.4	7.6	7.6	7.0	3.8
Phenylalanine & tyrosine	8.8	7.9	6.2	8.1	8.4	7.9	6.5	3.1
Threonine	5.4	4.9	4.2	4.4	4.2	4.5	3.5	2.8
Tryptophan	1.3	1.4	0.8	1.4	1.3	1.3	1.0	0.8
Valine	5.8	5.4	4.8	5.6	5.0	5.6	4.3	3.1

1. ILOB data
2. ICI data
3. National Academy of Sciences; Nutrient Requirements of Poultry (1971)
4. National Academy of Sciences; Nutrient Requirements of Swine (1968)

Table 3. Experiments required for nutritional and toxicological evaluation of SCP for animal feed

Evaluation		Test species: Laboratory animals		Target animals	
		Growth	Reproduction	Growth/ production	Reproduction
Nutritional aspects		-	-	+	+
Toxicity and safety aspects for	- Farm animals	+	+	+	+
	- Human consumers	+	+	+	+
	- Environment	-	-	+	-

Animal species react specifically to a feed and show differences in digestion, absorption, metabolism, accumulation, requirement for nutrients, and susceptibility to

toxins. Therefore, the choice of the species for the animal experiments is of crucial interest. For a nutritional evaluation, the target species provide clear and accurate answers. For safety evaluation for animals and for human consumers of the animal products, a full scale toxicological testing, including carcinogenecity and teratogenecity studies in rats is considered mandatory. In order to be specifically informed about safety aspects of SCP for farm animals and to be able to study the organoleptic and safety aspects of the animal products for consumers, researchers must obtain additional toxicological evidence from experiments using target species. Because of a possible specificity in reaction, these studies should include various stages of the life cycle such as reproduction, early growth, and the production phases.

Results presented in Table 4, show differences in digestibility of the same SCP in chickens and in pigs.

Table 4. Digestibility of SCP from fungal origin

	Digestibility coefficients[1]	
	chickens	pigs
Digestibility coefficient for:		
Organic matter	23.5	79.3
Nitrogen	59.3	71.0
Crude fat	18.1	34.2
Crude fiber	6.0	99.0
Nitrogen free extract	-	77.8
Metabolizable energy (kcal/kg) content	996	2940
Net energy content (kcal/kg)	-	1590

1. ILOB data

Several other small differences among animal species have also been observed many times during the experiments. The experimental results obtained with one batch of product are by no means transferable to other batches of the same product unless identical characteristics with regard to microorganism, substrate and process conditions are assured.

Digestibility and metabolizable energy

During preliminary experiments, the researcher should assess the acceptability of an SCP by the animals, and also determine its effect on performance. Before large scale experiments with great degree of accuracy are designed, a determination of digestibility and metabolizable energy should be carried out. Tables 5, 6, and 7 present the results of such experiments in three species of animals using Candida tropicalis, grown on gas oil, (Toprina L); Candida lypolitica, grown on paraffins (Toprina G); and Pseudomonas, grown on methanol (Pruteen). The results are compared with those for soybean oil meal and fish meal.

Table 5. Digestibility coefficients for Single Cell Proteins in chicks

	Toprina L[1]	Toprina G[1]	Pruteen[2]	Soybean oilmeal[3]	Fish meal[3]
Digestibility coefficient for:					
Organic matter	76	79	--	74	88
Nitrogen	85	84	90	80	90
Crude fat	--	78	--	38	91
Nitrogen free extract	68	68	--	80	50
Metabolizable energy content (kcal/kg)	2550	3050	3020	2200	2900

1. ILOB data
2. ICI data
3. TITUS (1955)

Table 6. Digestibility coefficients for Single Cell Proteins in pigs

	Toprina L[1]	Toprina G[1]	Pruteen[1]	Soybean oilmeal[2]	Fish meal[2]
Digestibility coefficient for:					
Organic matter	92	92	90	83	86
Nitrogen	92	90	93	91	86
Crude fat	--	95	87	34	85
Nitrogen free extract	92	94	--	94	--
Metabolizable energy content (kcal/kg)	3640	3860	3720	3190	3190

1. ILOB data
2. Data of the Central Bureau for Livestock Feeding in the Netherlands

In chicks, all three SCP products show a high digestibility of protein, the same as with soybean oil meal and fish meal (Vanweerden et al., 1970 and 1972). Metabolizable energy is particularly high for the unextracted Toprina G and for Pruteen. In pigs, protein digestibility is higher for all three SCP than for soybean oil meal and fish meal. The same is true for metabolizable energy. Again, the unextracted yeast shows the highest metabolizable energy content, because of the easily digestible fat fraction. In the study on veal, a comparison has been made with soyflour and fish protein

concentrate instead of fish meal (Vanhellemond & Vanweerden, 1973). Protein digestibility, and metabolizable energy using the three SCP compare favorably with the conventional products. In particular, the values obtained for Pruteen are encouraging.

Growth and Reproduction

The results of these experiments are presented in Tables 8, 9, 10, 11 and 12. Toprina has been substituted for a mixture of soybean oil meal and fish meal in all the experiments. Rations have been equalized for net energy and for digestible nutrients as far as possible.

Table 7. Digestibility coefficients for Single Cell Proteins in veal calves

	Toprina L[1]	Toprina G[1]	Pruteen[1]	Soyflour[1]	Fish concentrate[1]
Digestibility coefficient for:					
Organic matter	84	82	91	74	82
Nitrogen	85	82	94	80	86
Crude fat	--	78	99	--	--
Nitrogen free extract	83	84	--	68	--
Metabolizable energy content (kcal/kg)	3170	3560	3914	2920	3150

1. ILOB data

Table 8. Broiler chickens: Weight gain and feed conversion

	Control		12% Toprina L[1]		Control		14% Toprina G[2]	
	abs.	%	abs.	%	abs.	%	abs.	%
Weight gain (g)	1538	100	1509	98.1	1604	100	1592	99.3
Feed conversion (kg feed/kg weight)	2.04	100	2.02	99.0	1.90	100	1.89	99.5

1. 3 x 6 x 15 birds per group
2. 2 x 6 x 15 birds plus 2 x 4 x 80 birds per group

Summary: No difference between the control and the experimental group was observed. (Vanweerden et al. 1969; Vanweerden et al. 1970).

Table 9. Layers: Egg mass and reproduction criteria

	Control		10% Toprina L		Control		14% Toprina G	
	abs.	%	abs.	%	abs.	%	abs.	%
Kg egg/100 hen-days	3.80	100	3.79[1]	99.7	3.49	100	3.57[3]	102.3
Fertility (%)	91.6		89.3 [2]		91.2		88.6 [4]	
Hatchability (% of fertile eggs)	85.4		86.2		86.0		88.1	

1. 3 x 240 layers per group (P, F_1, F_2)
2. 2 x 120 layers per group; undiluted semen
3. 1 x 240 divided by 3 x 75 layers per group (P, F_1, F_2)
4. 1 x 120 divided by 3 x 75 layers per group; undiluted semen

Summary: The data are summarized for three successive generations of birds (P-, F_1- and F_2 generation). The production does not differ between experimental and the control groups. Reproductive performance (fertility and hatchability) also did not show any significant difference. (Vanweerden et al. 1969; Vanweerden et al. 1971).

Table 10. Pigs: Weight gain and feed conversion

	Control		15% Toprina L[1]		Control		15% Toprina G[2]	
	abs.	%	abs.	%	abs.	%	abs.	%
Weight gain (kg)	73.8	100	75.3	102.0	88.2	100	88.2	100.0
Feed conversion (kg feed/ kg weight gain)	3.11	100	3.03	97.4	2.83	100	2.80	98.3

1. 3 experiments with respectively 18, 24 and 32 pigs per group
2. 2 experiments with respectively 32 and 40 pigs per group

Summary: Growth and feed conversion was better in the Toprina L group than in the control. Differences were statistically significant in more than one experiment. For Toprina G, no difference can be seen in performance when compared with the control group. (Shacklady & VanderWal, 1968; VanderWal et al. 1971; VanderWal 1972 and 1975).

Table 11. Pigs: Reproduction criteria

	Control abs.	%	10% Toprina L[2] abs.	%	Control abs.	%	10% Toprina G[3] abs.	%
Conception rate	97		93		96		97	
Litter size[1]	9.4		9.7		9.1		9.3	
Still born or died within 24 hours	1.0		1.2		1.1		0.8	
New born weight (grams)	1337	100	1219	91.2	1335	100	1270	95.1

1. Normal: stillborn piglets included
2. 7 x 13/24 sows per group ($P\text{-}F_6$)
3. 3 x 13/18 sows per group ($P\text{-}F_2$)

Summary: Reproduction results of experiments with Toprina L have been presented for a parent and six filial generations. For Toprina G experiments, the second filial generation has been reached. The differences between the groups with regard to conception rate, litter size, number of stillborn animals, and newborn weight of the piglets have been small.

Table 12. Veal calves: Weight gain and feed conversion

	Control abs.	%	10% Toprina L[1] abs.	%	Control abs.	%	10% Toprina G[2] abs.	%
Weight gain (kg)	127.0	100	124.3	97.9	122.3	100	117.6	96.2
Feed conversion (kg feed/ kg weight gain)	1.48	100	1.50	101.4	1.53	100	1.55	101.3

1. 3 x 10/15 calves per group
2. 11 x 10/15 calves per group

Summary: Performance at this level of yeast inclusion is similar to that of the control-fed animals. At higher levels (results not shown) problems seem to be related to a poor carbohydrate digestion (Gaillard & Vanweerden 1976).

These reproduction experiments have been limited in size because of the great variability of the criteria. Moreover, a genetic drift may occur during the successive generation.

The results of the reproduction experiments mentioned above have been partly verified in an experiment with the two groups of 35 litter mate gilts. Five weeks after conception, the sows were killed. Conception rate, number of corpora lutea, and the sites with partly resorbed embryos were counted. Embryos were weighed. For none of these criteria have there been differences between the control group and the group fed 10 per cent Toprina (VanderWal & Shacklady, 1968; VanderWal et al., 1971; and VanderWal 1972 and 1975).

The relatively high level of iron in the yeast, compared with that in the replaced skim milk powder of the control ration, may adversely affect the desired whiteness of the meat. It is possible to lower the Fe-content of the yeast during SCP production.

Safety in farm animals

Apart from the above-mentioned records, possible toxic and organoleptic effects have been studied in most experiments. The state of health of the animals under study has been controlled daily by a veterinarian, and has not shown differences among groups. Blood characteristics and urine composition have been investigated. Carcass grading has been carried out at slaughter after prolonged SCP feeding. Organs and carcasses have been judged microscopically immediately after slaughter. Samples have been taken for further histopathological and chemical analysis of tissues and organs. No differences between Toprina-fed groups and controls were found with respect to any of the above criteria. (van der Wal, 1962 and 1965).

Discussion

The results obtained with the SCP's in ILOB experiments using laying birds and pigs have been confirmed in field trials elsewhere (Barber et al., 1971; Schiller et al., 1972; and Shannon & McNab, 1973).

Kirchgessner & Roth (1972) have included up to 10 per cent Toprina G in milk replacers for veal calves. At the highest level, a slightly negative effect on performance has been seen in the first part of the growing period. In the second part, growth was equal at the 0 and 10 per cent inclusion rate. In experiments with chickens, the results of the experiments depend strongly on the dosage applied. Waldroup et al. (1971) had the same experience with Toprina up to 10 per cent of inclusion. The Münchener group (Gropp, 1975, a and b: Beck, Gropp, 1974; and Tiews, 1974) has achieved optimum performance with Toprina up to a 15 per cent inclusion rate. All these authors have reported a growth depression at higher levels of inclusion, which cannot be completely overcome by special precautions.

At ILOB, a series of experiments has been carried out with broilers fed rations in which up to 28 per cent of Toprina has been included. A growth retardation was observed in these experiments. However, the growth retardation at this level of inclusion was overcome in three steps: a. pelleting of the feed; b. addition of 0.2 ppm Se; c. addition of 0.4 per cent arginine.

Table 13. Chickens: Influence of particle structure of feed

	Rations fed as meal[1]						Rations fed as pellets[2]			
	0		28% Toprina G (particle size 45 µ)		28% Toprina G (particle size 1000 µ)		0		28% Toprina G	
	abs.	%	abs.	%	abs.	%	abs.	%	abs.	%
Weight gain (g)	1077	100	970	90.1	1010	93.8	1072	100	1056	98.5
Feed conversion (kg feed/kg weight)	1.68	100	1.76	104.8	1.73	103.0	1.68	100	1.71	101.8

1. 3 x 6 x 15 birds per group; duration 5 weeks. Yeast ration with 0.2 ppm Se + 0.4% L-arginine.
2. 5 x 6 x 15 birds per group; duration 5 weeks. Yeast ration with 0.2 ppm Se + 0.4% L-arginine.

The effect of particle size of the yeast and of pelleting feed, including yeast, is shown in Table 13. The structure of the feed plays a predominant role in the growth depression. With increasing particle size of Toprina, the growth-depressing effect was reduced from 10 to 6 per cent. By feeding the rations as pellets, growth depression further disappeared. The experiments regarding the effect of Se and arginine addition will be published elsewhere. In the rations referred to in Table 13, both nutrients were added at levels mentioned above.

Summary: It may be concluded that the evidence presented illustrates that if an SCP is produced under well-controlled, sharply-defined conditions, it can successfully be substituted for conventional feed proteins. No harmful physiological effect has been seen in studies on a rodent (rat), an avian species (poultry), or a major mammal (pig) during the successive stages of the life cycle.

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DEVELOPMENT OF TECHNOLOGY AND EXPERIENCE IN THE USE OF SINGLE CELL PROTEIN IN ANIMAL FEEDING IN THE USSR

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Intensive research on the problem of single cell protein production from petroleum, and development of the technology for SCP industrial production began in the USSR, as in other countries, in the first half of the nineteen-sixties. During this time, the USSR produced SCP in the form of fodder yeast obtained from hydrolysates of plant materials -- industrial wastes from agriculture, food, timber, paper, and wood processing. The hydrolysis industry was created in the USSR as early as the mid-thirties -- i.e., long before the problem of SCP production was formulated as it has been since the late fifties. Hydrolysis production still exists in the USSR, but as a sub-branch of the microbiological industry. Four decades of existence of the hydrolysis industry has shown that development of SCP production for the needs of the animal husbandry could greatly expedite technical progress in agriculture and could considerably increase the efficiency of the economy.

The present need of the USSR in feed protein amounts to several million tons annually. Of course, a great part of this requirement is met from national agricultural sources and the fishing industry. However, it does not seem possible to satisfy this requirement fully just by the above-mentioned sources and the hydrolysis industry. This fact has given rise to the industrial implementation of the single cell protein production from petroleum hydrocarbons.

The possibility of the use of hydrocarbons for growing microorganisms was suggested for the first time in the USSR as early as 1926, by V.O. Tausson, who studied the bioenergetics of hydrocarbon oxidation by

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microorganisms and calculated a possible economic coefficient of biological utilization of hydrocarbons. In 1939, T.A. Tuasson, for the first time, isolated yeast organisms effectively oxidizing hydrocarbons. Neither in 1939 nor in 1926, could such work find any practical application, because of the level of development of technical microbiology and chemical technology and the level of available equipment and machinery necessary to set up the industrial production of single cell protein from petroleum. The solution of the specific problems in production became feasible only during the nineteen-sixties, with the advances in science and technology leading to intensive research and development in SCP and its industrial production from hydrocarbons.

One of the most important tasks related to SCP production from petroleum has been the selection of microorganisms suitable for this purpose. Hydrocarbons can be utilized by microorganisms of various systematic groups: bacteria, fungi, actinomycetes, and yeasts. Culture of the producing microorganism in quantity is one of the constituents of the complex system of the technological process, and represents - along with the equipment, chemicals and energy - an important production element. The efficiency of the microbiological production is to be determined by the productivity of the culture used, and the extent to which the technology conforms to the major physiological properties of the production strain of the microorganism. Only in such a case is a stable functioning of the whole system possible.

The major criteria of strain selection for biomass production are growth rate and economic yield coefficient of hydrocarbon assimilation. The following factors are also important:

- domination of the selected producing strain population;
- strain resistance to metabolites;
- norm of the strain response to the changing conditions of cultivation;
-maximum content of protein, essential amino acids, and vitamins in the cell;
- size and shape of the cell;
- thermal tolerance of the strain;
- safety of biomass for animals and non-pathogenicity of the strain to humans.

Features which characterize technologically valuable properties of the strain are closely interrelated, and the character of the relation can be different. Depending upon peculiarities of the technology, any of these features may prove to be the most valuable.

Taking into account the experience of researchers on yeast production on non-edible substrates and existing methods of yeast isolation, strains of Candida genus yeasts were used to develop the technology of single cell protein production.

Extensive research on isolation from natural substrates, and selection of the most active hydrocarbon oxidizing yeast, allowed researchers to create a fund of productive strains related to various species of Candida genus and ensure a technically acceptable flow rate of the medium and sufficiently high content of total protein. These strains made it possible to set up in the USSR as early as 1963, experimental industrial single cell protein production from hydrocarbons, in an experimental plant, specially built for this purpose, with a production capacity of 15,000 t/y. The same cultures became a basis for further selection of production strains. Construction of this plant, small by present standards, was by that time a big scientific and technical achievement, and the plant, itself, became a prototype for our modern plants.

During the study of the physiological peculiarities of hydrocarbon-oxidizing yeasts of Candida genus, of the selection under industrial conditions, and of the application of continuous culture method for selection, it was established that the selected yeasts possessed a high competitive ability in comparison with other species of Candida genus when grown on media with hydrocarbons.

The competitive ability of the selected yeasts with respect to other species of Candida was determined by the fact that metabolic products of the former inhibit development of other species. Strains of this type possess some other technologically valuable features, in particular, a wider norm of response to changes of some parameters of cultivation, viz: pH of the medium, and concentration of nitrogen, sulphur, and other components of the medium. They are more stable and less sensitive to the aeration conditions.

Application of continuous culture methods for selection of production strains and the use of selective media have helped to obtain fast-growing, competitive, spontaneous and induced mutants of Candida yeasts with a higher growth rate than that of the parent strains. Strains with a total protein content in the biomass of an average of 65 per cent (instead of 52-58 per cent in the parent strain) and with a higher methionine content have been obtained. In addition, all other economically valuable features have been preserved. The selected strains provide the continuous culture process with an economic coefficient of substrate utilization up to 120 per cent. Thus, at present, the USSR possesses a fund of highly productive yeast strains for single cell protein production.

A further increase of production efficiency can be reached by selection of new production strains having the following features: decrease of oxygen demand, increase of the economic coefficient of substrate utilization, and the content of protein and sulphur-containing amino acids in biomass, as well as others mentioned above.

The second, but not less important task is the choice of raw material for single cell protein production. Choice of specific fraction of the oil products for this purpose is determined by the presence of n-alkanes, because presently used microorganisms actively utilize only these hydrocarbons and have a high enough growth rate for industrial production. In addition, the use of different kinds of hydrocarbons for SCP production is dictated by their cost and requirements.

The possibility of producing SCP on an industrial scale on the basis of n-alkanes alone, combined with the desire to obtain the purest product by the simplest process, determined the choice of purified liquid n-paraffins produced from oil and purified by well known methods, as the most suitable raw material. That is the reason why this material was selected in the USSR at the beginning of the problem development.

Research on the individual n-alkanes utilization kinetics for the selected yeasts have shown that n-paraffins of the carbon number 11 and higher are most suitable for microbiological synthesis of SCP. Such paraffins are mainly isolated from the corresponding oil fraction with the help of molecular sieves, though they can be isolated by other methods.

The use of paraffins having a purity of no less than 99 per cent for SCP production and also the application of special processing techniques, permit production of fodder yeasts with a residual hydrocarbon content of no more than 0.1 per cent, which complies with USSR health requirements.

Yeast cultivation (traditionally called "fermentation") is the basic process which determines both economic efficiency and quality of the end product. The main criterion of the process, economic efficiency, is dependent on the productivity of a fermentation unit of time (per unit of fermenter volume), which, in turn, is determined by the economic coefficient of substrate utilization for the producing microorganism, mass transfer and hydrodynamic characteristics of the fermentation equipment and transport efficiency of nutrients and oxygen to the cells of a microorganism. The economic coefficient of substrate utilization is determined by both the quality of a hydrocarbon and the choice of operation conditions, which provide the optimal ratio of catabolic and anabolic activities of cells of a given strain.

Both the first Soviet pilot plant and the experimental production plant (of twelve thousand t), which were put into operation

in 1968, were equipped with fermenters of the air-lift type which are widely known in the world practice.

Nevertheless, the operational experience with the plant of twelve thousand t capacity --at that time, the first of this kind in the world--contributed much to the industry.

First of all, SCP for feed purposes was industrially produced in quantities sufficient to conduct extensive medical, biological and agricultural tests on the new product, which will be considered in detail later. Second, different features of the technology developed were revealed, in particular, the necessity of new development in order to increase the productivity of a fermentation unit.

The process of SCP production from n-paraffins accepted at the presently operating plants in the USSR consists of the following basic stages:

- reception and storage of the raw material and auxiliary materials;
- preparation of solutions of nutrient salts;
- growing of inoculation material;
- fermentation itself (production of yeast biomass);
- isolation, concentration and drying of biomass;
- storage and dispatching of the final product.

Solutions of salts and microelements are prepared in a special installation and then progress to the stage where inoculation material is grown, and then to the fermentation stage. The fermentation is carried in the intensive mass transfer equipment.

One of the most important indices of final product quality is the residual hydrocarbon content, and, therefore, one of the most important tasks of the technology is the reduction of their content in biomass to a minimum level.

Research on hydrocarbon localization in yeast cells has shown that during assimilation of n-paraffins by cells, hydrocarbons are spread on the cell surfaces, and in their walls and protoplasm; and the total hydrocarbon content in biomass is determined by the composition of the source paraffins, aeration conditions, pH and temperature. Paraffins with appropriate composition and a high degree of purity provide the production of biomass with a residual hydrocarbon content not higher than 0.1 per cent. The produced yeast suspension is transferred to the evaporation and drying stages. The dried yeasts, then, are stored at the final product store.

The technological system considered above is essentially a classical one for microbial biomass production processes. Problems arising in the development of the large-scale production technology were not at all the problems of compiling a suitable process flow-sheet. The regularities of the course of the processes had to be studied, and their optimum parameters for each specific stage had to be revealed in order to study the interrelation and interdependence of all the individual stages and put them together in such a way that the best technical and economical indices of the whole production process would be provided. These studies required a large volume of specific research applied to conditions of the large-scale production, since the experience of operating relatively small plants in the hydrolysis industry was not sufficient.

The second extremely important problem was, and still remains, the obtaining of producing strains which satisfy a number of indices. This work should be carried on continuously while production exists. This requires broadening genetic research and developing new selection and genetic methods for the production of economically valuable forms of microorganisms.

An exceptionally important problem of large scale production is the creation of an efficient computer control system on the basis of its mathematical modeling. Presently known mathematical models of the cultivation process usually consist of not more than ten parameters. For small

plants and installations, this is probably enough, but process models for large-scale production should include more parameters. It is also necessary to solve the problem of continuous monitoring at the production stages, in order to detect and eliminate in time all possible deviations from the normal course of the process, and to ensure the required quality of the final product.

Basically, all these problems have been solved in the USSR. This has resulted in the creation of the large-scale production of fodder yeast from liquid normal paraffins.

The technological process developed and tested at the experimental production plants mentioned above was taken as a basis in design for the large capacity plants with a capacity of up to 250 thousand t. They were designed at the end of the nineteen-sixties and soon their construction began. The first of them was put into operation in 1973, and the others in '74 and '75. During the coming five years (1976 to 1980), the USSR plans to build several more large-scale plants for production of fodder yeast from liquid normal paraffins. All of their production is meant only for domestic consumption, since the demand of animal husbandry in the USSR for feed protein is very great.

It should be emphasized that the technology and equipment have been completely designed by Soviet scientists and engineers. That is, the Soviet Union has not only its own technology and equipment but its own stable, large-scale production. At the International Symposium on Single Cell Protein held in Rome on November 7-9 1973, it was stated in a report ("Economics of Single Cell Protein in Relation to World Protein Supplies" Symposium proceedings edited by P. Davis, Academic Press, 1974, pp. 25-45), that by 1973, the USSR had only one operating plant, with a capacity of 16,000 t/y, built by the British company Messrs. Rosedown. There has never been such a plant in the USSR. If the USSR ever showed interest in purchasing technologies and equipment from other firms, this was done for the sake of achieving the quickest and fullest provision of USSR animal husbandry with single cell protein, and not

Table I.

General composition of fodder yeasts, fish meal and soybean cake

Indices (Percentage weight)	Fodder Yeasts		Fish Meal	Soybean Cake
	From N-paraffins	From Carbohydrates (hydrolysis)		
Moisture	6-10	6-10	9-12	10
Total protein: (N x 6,25) on a dry matter basis	60-65	50-60	48-62	46
Lipids	up to 5	0.3-4.0	2.0-11.0	1.5
Carbohydrates	15-20	11-23	-	28

One can see that the quality of fodder yeasts produced from liquid n-paraffins is as good as protein products from fish meal in terms of the most important index-protein content. The same can be said about amino acid composition.

because the USSR does not have its own operating processes and equipment.

The fodder yeasts produced in the USSR from liquid paraffins differ very little in their basic composition from yeasts produced from other types of raw materials. This can be seen in Table I, in which the fish meal and soybean cake composition are also included for comparison.

As to the possible content of deleterious heavy metals (lead, mercury, cadmium and others--a problem that lately is worrying some specialists and even official agencies of some countries), the practice of the Soviet industry has shown that if the raw material, mineral salts, and technological water used in production have the required purity, and the process is conducted in strict accordance with the requirements of the technology, there is no need to check the content of these possible contaminants in all the intermediate products and in the end product. (This should be done at the stage when the raw materials and auxiliary materials are checked.) It has been established by special analyses that the fodder yeasts produced in the USSR from liquid n-paraffins meet the requirements for the admissible heavy metals content for food products.

Numerous and extensive medical and biological tests which have been conducted in the USSR for a period of more than thirteen years have shown that the yeast produced from n-paraffins which complies with the quality requirements is absolutely safe for animals, and has neither toxic carcinogenic, leukogenic, teratogenic, nor any other deleterious effects, whereas its high biological value remains unaffected.

Numerous and extensive tests, which have also been carried out in agriculture, have shown that digestibility of the n-paraffin fodder yeast for various types of animals is about 85 per cent. These tests have proven the safety of the yeast for animals, as well as the safety of food products obtained from these animals. It has been demonstrated that addition of the feed yeast to rations of animals and birds stimulates their growth, development, and productivity.

Use of the fodder yeast produced from n-paraffins in the rations of pigs increases the average daily weight gain of the animals by 25-30 per cent, reduces feed consumption by no less than 20 per cent per one center of weight gain, increases product output by a quarter per one thousand feed units, and shortens fattening time of pigs by a month or even more. Addition of the fodder yeast from n-paraffins to mixed feeds for broilers results in an additional weight gain of 120-125 kg for one ton of the feed. Use of mixed feeds enriched with the yeast of n-paraffins in the rations of laying hens increases the number of eggs by 20-25 per cent, and reduces feed consumption by nearly 30 per cent. Use of the yeast from n-paraffins for feeding calves and piglets from an early age considerably reduces the amount of cow's milk they must be fed, and thus makes this valuable product available for human consumption.

The yeast from n-paraffins presently produced in the USSR is used as a protein component of mixed feeds made at state mixed feed plants, as well as at plants and shops of collective farms, state farms and agro-industrial complexes. It is also added to feeds right at animal husbandry farms. The application of the yeast from n-paraffins in animal husbandry confirms the experimental research conclusions about its high efficiency. At the same time, the great demand of the Soviet national economy for protein has necessitated a search for both new types of feed stock and new microorganisms for protein production.

A study of the physiological characteristics of microorganisms of various systematic groups able to assimilate hydrocarbons has shown that the most promising, from the standpoint of such features as productivity, protein content in biomass, and others, are bacteria and microorganisms of the mycococcus genus, which have considerably higher growth rates on media with hydro-

carbons than yeasts of Candida genus. They also have higher economic coefficients of substrate utilization with lower oxygen demand, and contain up to 75 per cent of the total protein in the biomass.

However, the small cell size of the bacteria hampers their isolation from the culture medium under industrial conditions, which makes it necessary to develop totally new methods for concentrating microbial suspensions.

With regard to new types of raw materials, laboratory research and tests of the existing processes at experimental and pilot plants show that it would be quite feasible to use such types of raw material as methyl and ethyl alcohols, oil distillates, natural gas and, probably in the future, crude oil for single cell protein production.

Development of technological processes for SCP production from these types of raw material is progressing rapidly in the USSR, and in the course of the coming five years (1976-1980) some of these processes are planned for implementation on the scale of experimental production plants.

To conclude this report, it should be noted that the establishment and development of large scale SCP production for animal feeds from petroleum in the USSR has made a great contribution to the economy of the country.

BIOLOGICAL SIGNIFICANCE OF UNEVEN FATTY ACIDS

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Single cell proteins (SCP) produced by growing Candida maltosa* on linear paraffins (C14-C20), like other SCP, contain relatively high concentrations of uneven fatty acids (mostly C15 and C17), usually considered non-physiological for the animal species used as human food. In reality, uneven fatty acids (UFA) are widely present in natural lipids in concentrations ranging from 1 to 5% of the total fatty acids, and several foods of animal origin (i.e., fish, bovine milk, human milk, fat lamb) contain UFA in relatively large amounts (18-33). Mammalian species have the biochemical mechanism to metabolize them (24).

This report summarizes a number of investigations in several animal species to test whether a diet containing SCP in various amounts results in an increase of UFA in animal tissues and if so, whether this increase might be responsible for toxic effects not detectable in routine toxicological studies. This report is a brief account of a large scale investigation, still in progress.

Uneven fatty acids (UFA) content of SCP

The SCP selected for this study (Liquipron), contains 12.7% lipids and uneven fatty acids represent 3.5% of the total weight.

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*The yeast Candida maltosa (Liquipron^{R}) produced by Liquichimica Biosintesi S.p.A., Milan, Italy, has the following percentage composition: moisture 4.8, crude protein 59.7 $\pm$ 0.2; Lipids (Chloroform: methanol 2:1 extraction) 11.0 $\pm$ 0.2; Fibers 4.7 $\pm$ 0.2; Ash 10.8 $\pm$ 0.3; Nitrogen-free extract 13.9 $\pm$ 0.4 (figures are means $\pm$ S.E. of 10 batches).

The profile of the fatty acids in Liquipron is given in Table 1.

Table 1.

Fatty acid profile of the total lipids of SCP (Liquipron)

Fatty acid (a)	Percent of total fatty acids
14:0	1.5
14:1	0.2
15:0	3.6
15:1	0.4
16:0	10.6
16:1	10.6
16:2	tr.
17:0	6.3
17:1	27.9
17:2	tr.
17:3	3.1
18:0	1.3
18:1	19.8
18:2	11.9
18:3	2.6
Even	58.5
Uneven	41.3

a: carbon number of chain and number of double bonds.

The ratio between saturated and unsaturated is 0.3 and the ratio between monoenes and polyenes is 3.3. This product was incorporated into diets for rats so that 20, 50 or 80% of the protein of the control diet was substituted with SCP and the composition of the resulting pellets is shown in Table 2.

Effect of SCP on rat lipids

Male and female CD rats (Charles River, Italy), initial average weight of 66 $\pm$ 2 g, were fed for up to 15 months with diets in which 20, 50 and 80% of the protein content had been substituted with SCP. At the end of treatment the animals were killed for determination of the lipid fractions in plasma and in several tissues including adipose tissue, heart, kidney, liver, brain and adrenals. Lipids were extracted with chloroform-methanol 2:1 as described by Carlson (6). Aliquots of the chloroform phase were used to measure triglycerides by Van Handel and Zilversmit's method (14), phospholipids according to Svanborg and Svennerholm (36) and cholesterol by the Lieberman Burchard reaction. Plasma free fatty acids (FFA), corticosterone and 3-hydroxybutyric acid were determined according to Trout et al. (7), Guillemin et al. (13) and Young and Renold (40) respectively.

Table 2.

Composition of diets

	C %	L20 %	L50 %	L80 %
Proteins	18.33	18.36	18.35	18.32
Fat	5.80	5.67	6.70	6.98
Nitrogen free extract	54.0	55.4	55.5	52.4
Fiber	3.7	3.5	3.4	3.6
Ash	7.8	7.5	7.0	7.2
Calories	341	346	356	347
Uneven fatty acids		0.24	0.57	0.92

In this and subsequent tables, L20, L50 and L80 indicate protein of basal diet substituted with Liquipron proteins by 20, 50 and 80% respectively. C refers to control group.

When SCP replaced 20% and 50% of dietary proteins there were no changes in the lipid classes mentioned. Only at the 80% SCP dose was there a significant decrease in liver triglycerides and adrenal cholesterol and an increase of β-OH butyric acid, while all other parameters remained essentially similar to the group of controls (Table 3). It was concluded that the high levels of UFA in SCP did not affect the concentrations of the major lipid classes in plasma and in several tissues in rats, except when fed in high doses.

Effect of SCP on the fatty acid profile of total lipids of adipose tissue

In another series of experiments male and female rats given a diet containing 80% of the protein as SCP were killed at various times and the fatty acids composition of the epididymal or parametrial adipose tissue was determined. Tissue lipids were extracted according to Folch (10). Transmethylation of fatty acids was performed using methanol - sulphuric acid (5%) at 80°C for 60 min, in the

Table 3.

Plasma and tissue lipids of male (M) and female (F) rats fed SCP diets for 3 months

	Controls		L 20		L 50		L 80	
	M	F	M	F	M	F	M	F
PLASMA								
β-OH butyric acid µMol/100 ml ± S.E.	15 ± 1	31 ± 1	16 ± 1	45 ± 7	25 ± 2	51 ± 1	42 ± 16	66 ± 2
Triglycerides mg/100 ml ± S.E.	93 ± 9	71 ± 6	136 ± 9	75 ± 9	98 ± 9	60 ± 11	94 ± 2	62 ± 5
Cholesterol mg/100 ml ± S.E.	81 ± 7	112 ± 9	73 ± 4	96 ± 8	86 ± 6	120 ± 7	90 ± 5	117 ± 7
Phospholipids mg/100 ml ± S.E.	92 ± 7	132 ± 7	100 ± 5	135 ± 10	95 ± 5	160 ± 12	97 ± 5	145 ± 5
F F A µEq/l ± S.E.	347 ± 4	508 ± 55	265 ± 40	518 ± 55	382 ± 36	519 ± 85	346 ± 74	537 ± 67
LIVER								
Triglycerides mg/100 g ± S.E.	782 ± 111	544 ± 69	562 ± 75	502 ± 44	556 ± 101	630 ± 94	394 ± 44	468 ± 50
Cholesterol mg/100 g ± S.E.	282 ± 18	274 ± 8	259 ± 18	266 ± 6	281 ± 15	271 ± 9	235 ± 12	266 ± 8
Phospholipids mg/100 g ± S.E.	2850 ± 150	2500 ± 75	2550 ± 100	2600 ± 75	2850 ± 150	2575 ± 50	2825 ± 75	2475 ± 100
HEART								
Triglycerides mg/100 g ± S.E.	98 ± 12						86 ± 5	
Cholesterol mg/100 g ± S.E.	157 ± 6						166 ± 10	
Phospholipids mg/100 g ± S.E.	1975 ± 50						2050 ± 50	
ADRENALS								
Cholesterol mg/g ± S.E.	73.5 ± 0.7	73.3 ± 0.4	71.3 ± 0.9	65.7 ± 0.7	64.7 ± 0.5	42.3 ± 0.3	66.6 ± 0.7	53.0 ± 0.8

presence of small amounts of hydroquinone to avoid peroxidation (4). The methylesters, extracted with petroleum ether, were separated by thin layer silica gel chromatography with petroleum ether: diethylether: methanol: acetic acid (90:7:2:0.5) in order to exclude other lipids or interfering compounds, then extracted with ethylether, evaporated and redissolved in heptane. The fatty acids composition of the methylesters was analyzed on a gaschromatograph (Fractovap 2350, Carlo Erba) fitted with a flame ionization detector and dual columns packed with 15% DEGS on Chromosorb W; nitrogen was used as carrier gas (flow rate 33 ml/min), the detector and injector temperatures were 195°C and 275°C.

Table 4 shows the pattern of fatty acids in controls and in SCP treated rats at different ages. Since age changes the fatty acids profile, the data obtained after feeding SCP is compared with controls of the same age. UFA were also found in the adipose tissue of control rats, accounting for a minumum of 0.4% of the total fatty acids (15 months of age) and a maximum of 2.6% (2 months of age). The SCP fed animals showed a maximum UFA content of about 10% after 2 months of feeding but this percentage did not increase with the duration of SCP administration. Due to the increase of

Table 4.

Fatty acids profile of adipose tissue from rats

Fatty acids	PERCENT OF TOTAL FATTY ACIDS							
	21 days		2 months		11 months		15 months	
	C	L 80	C	L 80	C	L 80	C	L 80
10:0	1.5	-	-	-	-	-	-	-
12:0	8.3±1.4	8.5±1.3	0.9±0.3	1.2±0.1	tr.	1.5±0.2	0.9	2.3
14:0	12.9±0.4	12.7±0.1	4.5±0.3	3.9±0.2	1.6±0.2	2.5±0.1	1.8	2.9
15:0	0.4±0.1	1.6±0.1	0.7±0.2	2.0±0.2	0.5±0.0	1.7±0.1	0.4	1.9
15:1	-	-	-	tr.	tr.	tr.	-	-
16:0	28.1±0.9	23.2±1.2	28.4±0.1	22.0±1.5	23.6±2.6	19.8±0.4	24.4	20.1
16:1	3.9±0.3	5.5±0.6	10.1±0.6	10.3±0.4	4.9±0.5	8.2±1.0	3.7	4.0
17:0	0.5±0.0	1.2±0.1	0.9±0.0	1.9±0.3	0.8±0.4	1.2±0.1	tr.	1.5
17:1	0.3±0.0	3.0±0.7	1.0±0.1	6.0±0.3	0.9±0.6	5.3±0.4	tr.	4.2
18:0	3.6±0.2	3.8±0.3	3.4±0.1	3.9±0.3	3.6±0.4	2.9±0.4	3.9	4.5
18:1	27.9±1.9	28.7±2.4	37.0±0.8	36.9±0.3	41.2±2.6	38.5±0.5	39.3	39.8
18:2	11.6±1.0	8.8±0.1	11.9±0.3	12.3±2.8	22.7±6	18.1±1.3	24.6	10.1
18:3	0.7±0.0	1.6±1.0	0.7±0.3	1.0±0.1	tr.	tr.	1.0	0.7
Uneven	1.2	5.8	2.6	9.9	2.2	8.2	0.4	7.6
Saturated	56	52	39	34	30	30	31	33
Monoenes	32	37	48	53	47	52	43	48
Polyenes	12	10	13	13	23	18	25	11

Treatment schedule of 21 days and 2 months old rats was: pregnant rats received diet containing SCP 10 days before delivery, the treatment continued during lactation and after weaning was given to the littermates. The 11 months and 15 months treatment began after weaning.

Mean ± S.E. of 4 rats in each group.

adipose tissue with age, the total amount of UFA present in the body was actually greater in the old than in the young animals. No new UFA appeared in the SCP treated rats compared with the controls. C 17:1 was the UFA which increased most after SCP treatment, followed by C 17:0 and C 15:0. The ratio between unsaturated and saturated fatty acids remained comparable in the control and treated rats with a clear, age-dependent increase. Other experiments summarized in Table 5 indicate that the increase of UFA in rat adipose tissue was proportional to the concentration of SCP in the diet. The percentage of UFA in total fatty acids ranged from 3.7% in the adipose tissue of rats fed L20 up to 5.6% and 7.6% in L50 and L80 when the diets were given for 15 months.

Table 5.

Uneven fatty acid present in adipose tissue of rats

Duration of treatment	UNEVEN FATTY ACIDS (percent of total fatty acids)			
	controls	L20	L50	L80
3 weeks	1.2	-	-	5.8
2 months	2.6	2.5	4.1	9.9
3 months	1.6	-	-	5.2
11 months	1.4	-	-	8.2
15 months	0.4	3.7	5.6	7.6

The reversibility of the fatty acid pattern of SCP treated animals upon withdrawl of the

Table 6.

Fatty acid profile of total lipids in rat adipose tissue

Fatty acid	PERCENT OF TOTAL FATTY ACIDS		
	Controls	L 80	L 80 withdrawn
12:0	0.9 ± 0.3	1.2 ± 0.1	0.9 ± 0.1
14:0	4.5 ± 0.3	3.9 ± 0.2	3.0 ± 0.1
15:0	0.7 ± 0.2	2.0 ± 0.2	0.4 ± 0.1
15:1	tr.	tr.	tr.
16:0	28.4 ± 0.1	22.0 ± 0.5	27.1 ± 1.5
16:1	10.1 ± 0.6	10.3 ± 0.4	9.6 ± 0.9
17:0	0.9 ± 0.0	1.9 ± 0.3	0.7 ± 0.1
17:1	1.0 ± 0.1	6.0 ± 0.3	0.7 ± 0.0
18:0	3.4 ± 0.1	3.9 ± 0.3	3.4 ± 0.1
18:1	37.0 ± 0.8	36.9 ± 0.3	35.1 ± 0.9
18:2	11.9 ± 0.3	12.3 ± 2.8	17.8 ± 1.0
18:3	0.7 ± 0.3	1.0 ± 0.1	0.8 ± 0.1
Uneven	2.6	9.9	1.8
Saturated	39	34	36
Monoenes	48	53	45
Polyenes	13	13	19

Treatment schedule as in Table 4. When L 80 was withdrawn, rats received control diet.

Mean ± S.E. of 4 rats in each group.

diets containing SCP is shown in Table 6. In animals given a 2-month treatment with diets containing 80% of the protein as SCP, the elevated UFA concentrations returned to control levels when a normal diet was given for a period of one month. The findings that the accumulation of UFA was proportional to the concentration of SCP in the diet and that the UFA level did not increase with the duration of feeding and returned to normal when the animals were put back onto control diets favor the hypothesis that UFA can be metabolized by rats.

Effect of SCP feeding on fatty acid pattern of other animal species

This was studied in the mouse (parametrial), the chicken (subcutaneous) and the monkey (macaca mulatta) (subcutaneous) (Table 7). As in rats, the greatest increase was seen in C17:1, followed by C17;0 and C15:0 in the three species. The ratio of unsaturated to saturated fatty acids also remained similar in controls and in SCP-treated groups. Table 8 showing the increase of UFA in the adipose tissue of the various animal species fed SCP failed to reveal any significant interspecies difference in reacting to a diet containing a relatively high concentration of UFA.

Functional significance of UFA in the adipose tissue

Mammals can utilize UFA by the usual pathway of beta-oxidation (24). The last 3 carbon atom fragment is bound to coenzyme A (CoA) and carboxylated by a propionyl carboxylase to form methylmalonylCoA which in turn is metabolized to succinylCoA via a methylmalonyl mutase (33) (see Figure 1 at end of text). The succinyl CoA enters the normal tricarboxylic acid cycle (3). In some pathological conditions, difficulty in metabolizing

Table 7.

Fatty acid profiles of total lipids in mouse, chicken and monkey adipose tissues

Fatty acids	PERCENT OF TOTAL FATTY ACIDS						
	MOUSE		CHICKEN		MONKEY		
	Controls	L 80	Controls	L 50	Controls	L 30	L 80
12:0	0.4 ± 0.1	2.4 ± 0.5	tr.	tr.	-	-	-
14:0	2.9 ± 0.4	4.4 ± 0.6	1.3 ± 0.2	0.7 ± 0.4	2.0	1.7	3.5
15:0	0.2 ± 0.1	1.6 ± 0.1	0.2 ± 0.1	1.0 ± 0.1	0.2	0.4	0.8
15:1	tr.	tr.	tr.	tr.	-	-	-
16:0	25.6 ± 3.5	25.2 ± 1.1	26.1 ± 1.2	20.9 ± 1.3	29.7	27.4	27.0
16:1	9.8 ± 0.8	13.1 ± 0.8	3.8 ± 0.1	5.3 ± 0.2	4.2	4.8	10.5
17:0	1.0 ± 0.1	1.5 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.8	1.2	1.2
17:1	0.5 ± 0.1	4.0 ± 0.1	tr.	2.4 ± 0.2	0.5	2.2	3.9
18:0	3.7 ± 0.2	2.8 ± 0.2	11.3 ± 0.3	9.5 ± 0.4	13.3	15.4	8.0
18:1	37.7 ± 2.3	36.7 ± 1.3	37.5 ± 1.5	37.1 ± 2.3	37.6	36.7	35.1
18:2	19.3 ± 2.7	8.2 ± 0.5		20.5 ± 0.1	10.6	9.6	9.5
18:3	1.7 ± 0.3	0.4 ± 0.2		1.1 ± 0.1	tr.	tr.	tr.
Uneven	1.7	7.1	0.8	4.2	1.5	3.8	5.9
Saturated	31	38	40	34	47	47	41
Monoenes	48	54	41	45	42	44	50
Polyenes	21	9	19	21	11	10	10

Treatment schedule for mice was similar to that described for the rat (Table 4); chickens received the diet for 12 months, monkeys for 6 months.

Mean ± S.E. of 4 animals in each group.

Table 8.

Uneven fatty acids present in lipids of adipose tissue of rat, mouse, chicken and monkey

Animals species (a)	Uneven fatty acids percent of total fatty acids		Unsaturated saturated fatty acids	
	C	SCP	C	SCP
Rat (21 d)	1.2	5.8	44/56	48/52
Rat (2 m)	2.6	9.9	61/39	66/34
Rat (11 m)	2.2	8.2	70/30	70/30
Mouse (12 m)	1.7	7.1	68/31	62/38
Chicken (12 m)	0.8	4.2	60/40	66/34
Monkey (6 m)	1.5	5.9	53/47	59/41

Diets were L 80 for rat, mouse and monkey, L 50 for chicken.
(a) in brackets: duration of treatment.

propionic acid results in the accumulation of methylmalonic acid (25, 26, 30). It was therefore of interest to establish whether animals treated with SCP differed from controls for the methylmalonic acid content in plasma. Rats were fed for 3 months with diets containing 80% of the proteins as SCP. At the end of the treatment animals were killed and plasma levels of methylmalonic acid were measured according to the method of Schiller and Summer (32). In both groups methylmalonic acid was not detectable in plasma, using a method with a sensitivity around 0.5 µg/ml. Whether conditions of stress induced during SCP feeding brought about any similar biochemical changes in the lipids was tested in a few experiments. The results obtained are summarized in Table 9. Exposure to cold induced a marked increase of plasma free fatty acids (FFA), 3-hydroxybutyrate and corticosterone, with a concomitant decrease of adrenal cholesterol. The intensity of these biochemical responses was similar for both control and experimental rats. The effect of prolonged physical exercise was tested in mice fed control experimental diets. After 2 months of feeding, animals were tested for their resistance to swimming according to the test developed in our laboratory and for their ability to balance without falling on a revolving bar (rotarod test) (38). The results obtained are summarized in Table 10; no differences were found between the control and experimental groups.

Fatty acid pattern in the heart of rats treated with SCP

A summary of the uneven fatty acid content of the heart of rats fed diets containing different amounts of SCP for differing periods of time is presented in Table 11. No significant changes in the UFA content occurred in the heart when animals were fed for 15 months with diets containing 20% of SCP. However when the SCP content was 50 or 80%, UFA reached concentrations of 2.6% and 3.2% respectively. Table 12 gives profile of fatty acids of total lipids from the hearts of rats fed with SCP (L80) for 2 months and 11 months. The fatty acids which were most affected were C17:0 and 17:1. The duration of the SCP feeding from 2 to 11 months did not substantially affect the UFA content, and there was no accumulation of UFA in the heart as a function of time of exposure to SCP. The fatty acids profile was also established for heart phospholipids and these are reported in Table 13.

In order to check whether the presence of UFA in the heart might have functional

Table 9.

Effect of cold exposure (18 hr) in rats fed diets containing SCP (for three months)

Diet	Exptl. Cond.	PLASMA FFA µEq/L ±SE	CS µg/ml ±SE	β-OH B. mol/l ±SE	Chol. mg/100 ml ±SE	TG mg/100 ml ±SE	P(lip) mg/100 ml ±SE	Liver TG mg/100 g ±SE	Adrenals Chol. mg/g ±SE
Control	Fasting	291±72	0.32±0.06	0.62±0.10	79±3	47±12	4.3±0.2	959±152	41.9±1.9
	Cold	686±30 a	0.50±0.03 a	2.08±0.24 a	92±2	33± 5	5.0±0.1	1254±126	21.2±1.6 a
L 20%	Fasting	388±45	0.34±0.08	1.38±0.15	69±7	45± 5	3.5±0.2	966±111	32.4±5.1
	Cold	881±73 a, d	0.47±0.09	3.43±0.51 a, d	101±5	28± 2	5.1±0.3	1114±160	14.3±1.0 a, c
L 50%	Fasting	496±33	0.41±0.06	1.35±0.17	86±1	43±8	5.1±0.1	850± 86	40.6±2.4
	Cold	928±61 a, d	0.57±0.04	3.19±0.22 a, d	97±3	24±1	6.4±0.2	911± 87 a, e	15.1±2.4 a
L 80%	Fasting	492±33	0.39±0.06	1.55±0.11	82±3	37±7	4.8±0.1	549± 58	29.7±1.7
	Cold	659±29 a	0.65±0.03	2.25±0.34	87±2	20±2	5.5±0.2	552± 63 d	10.3±1.6 a

a = $p < 0.01$ cold against fasting
b = $p < 0.01$ and c = $p < 0.05$ fasting (Liquipron against controls)
d = $p < 0.01$ and e = $p < 0.05$ cold (Liquipron against controls)

Table 10.

Behavior of mice fed SCP in the swimming test and rotarod test

Treatment	Sex	Swimming min ±SE	Revolving bar
Controls	M	5 ±0.3	>5'
L 20%	M	4 ±0.5	>5'
L 50%	M	5 ±0.3	>5'
L 80%	M	7 ±0.5	>5'
Controls	F	15 ±2	>5'
L 20%	F	19 ±1	>5'
L 50%	F	14 ±3	>5'
L 80%	F	15 ±2	>5'

Swimming test was performed at 17°C and mice were loaded with 0.5 g/10 g bw.

Treatment schedule as in Table 4.

Table 11.

Percent of uneven fatty acid in total lipids from heart of rats

Tissue	Duration of treatment (months)	C	Uneven fatty acids % of total fatty acids L 20	L 50	L 80
Heart	2	0.7	-	-	2.9
	3	2.9	-	-	4.2
	11	0.9	-	-	4.3
	15	1.9	2.3	2.6	3.2

Treatment schedule as described in Table 4.

significance, several parameters of cardiac function were studied. The heart rate was comparable in rats fed with control diet or with one containing 80% of the proteins as SCP. The ECG recorded in several derivations showed comparable morphology for

Table 12.

Fatty acids profile of total lipids in heart from rats

Fatty acids (a)	PERCENT OF TOTAL FATTY ACIDS			
	2 months		11 months	
12:0	tr.	tr.	0.2 ± 0.0	0.4 ± 0.1
14:0	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1
14:1	tr.	tr.	0.2 ± 0.1	tr.
15:0	tr.	tr.	0.2 ± 0.0	0.8 ± 0.0
15:1	tr.	tr.	0.2 ± 0.0	0.2 ± 0.0
16:0	18.2 ± 1.5	15.2 ± 0.2	18.3 ± 2.7	14.0 ± 0.3
16:1	1.2 ± 0.1	1.7 ± 0.1	1.4 ± 0.3	1.6 ± 0.1
17:0	0.7 ± 0.1	1.6 ± 0.0	0.4 ± 0.0	1.6 ± 0.1
17:1	tr.	1.3 ± 0.1	0.2 ± 0.0	1.7 ± 0.0
18:0	24.7 ± 0.8	23.2 ± 0.2	23.6 ± 2.8	24.5 ± 0.0
18:1	13.6 ± 0.9	15.6 ± 1.6	17.2 ± 1.8	17.6 ± 0.6
18:2	25.0 ± 1.6	21.3 ± 0.2	25.7 ± 1.6	19.6 ± 0.4
20:4	15.0 ± 0.3	17.7 ± 1.0	11.6 ± 0.1	16.5 ± 1.8
Uneven	0.7	2.9	1.0	4.3
Saturated	44.1	40.6	43.3	42
Monoenes	14.8	18.6	19.2	21.1
Polyenes	40	39.0	37.3	36.1

Treatment schedule as in Table 4.
(a) carbon number of chain; number of double bonds.
mean ± SE of 4 rats in each group.

both experimental groups. Administration of agents capable of increasing heart rate did not produce significant differences between the two groups. For instance, noradrenaline (3 µg/kg i.v.) increased heart rate in controls by 70 ± 20 beats/min as opposed to 83 ± 12 in SCP treated rats, while isoprenaline (1 µg/kg i.v.) increased heart rate by 109 ± 25 and 85 ± 14 beats/min respectively in the two experimental groups.

Fatty acids pattern in the liver of rats treated with SCP

Table 14 shows the pattern of neutral and polar fatty acids in the liver of rats treated with a control diet or with a diet containing SCP (L 80%) for a period of two months. In control livers the percentage of UFA content was 1.9 and 1.5 in neutral and polar lipids, respectively, while these figures were 7.6 and 6 for SCP treated animals. The highest increase in neutral lipids was in C17:1; followed by C17:0 and C15:0, while in polar lipids the highest percentage of uneven fatty acids was found in C17:0. On the whole UFA seemed to increase more in the neutral than in the phospholipids fraction; however since the concentration of phospholipids is far higher than the neutral lipids the absolute amount of UFA was higher for phospholipids. The ratio of unsaturated to saturated fatty acids was not affected by SCP for both classes of liver lipids.

Fatty acid pattern of liver microsomes of rats treated with SCP

Liver microsomes are a fraction obtained

Table 13.

Fatty acid profiles of phospholipids in rat heart

Fatty acids (a)	PERCENT OF TOTAL FATTY ACIDS 2 months (b) controls	2 months (b) L 80	11 months controls	11 months L 80
12:0	0.4 ± 0.2	0.2 ± 0.0	0.3 ± 0.0	0.8 ± 0.5
14:0	0.2 ± 0.0	0.2 ± 0.0	0.4 ± 0.2	0.4 ± 0.2
14:1	tr.	tr.	0.5 ± 0.0	tr.
15:0	0.2 ± 0.0	0.8 ± 0.1	0.2 ± 0.0	0.7 ± 0.2
15:1	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.1	0.2 ± 0.0
16:0	17.7 ± 1.9	12.8 ± 0.4	16.5 ± 3.0	18.4 ± 1.7
16:1	0.6 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.2 ± 0.1
17:0	0.5 ± 0.0	2.3 ± 0.4	0.5 ± 0.0	1.3 ± 0.1
17:1	tr.	1.2 ± 0.2	0.3 ± 0.0	1.3 ± 0.3
18:0	31.6 ± 1.9	21.7 ± 0.9	26.2 ± 1.5	25.7 ± 1.5
18:1	11.1 ± 0.3	15.3 ± 1.0	17.6 ± 1.7	13.6 ± 3.0
18:2	18.4 ± 1.7	19.4 ± 1.0	24.1 ± 0.6	19.7 ± 0.3
18:3	0.5 ± 0.3	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.0
20:0	0.3 ± 0.0	0.4 ± 0.0	0.2 ± 0.1	--
20:4	13.3 ± 2.5	17.1 ± 0.3	9.8 ± 0.8	15.9 ± 4.6
20:5 + 22:0	0.4 ± 0.1	0.4 ± 0.1	--	--
22:6 and others	4.6 ± 1.3	6.1 ± 0.6	--	--
Uneven	1.0	4.5	1.4	3.5
Saturated	50.9	38.4	44.3	47.3
Monoenes	12.0	17.7	19.7	16.3
Polyenes	37.2	43.0	34.2	35.8

Treatment schedule: 2-month treated rats received L 80 as described in Table 4; 11-month treated rats received L 80 after weaning.
Mean ± SE of 4 rats in each group.
a) carbon number of chain : number of double bonds.
b) gas chromatographic analysis of these groups was performed on columns packed with ASI 50 phenyl-50 cyanopropyl 3% on gas Chrom Q 80-100 with temperature programming in steps of 1°C/min from 160°C to 215°C.

after centrifugation at 105,000 x g, containing the tubular system of the liver endoplasmic reticulum system, a subcellular structure rich in cytochromes and enzymes involved in the metabolism of endogeneous and foreign compounds (23). Table 15 shows the profile of total fatty acids present in the liver microsomal fraction of rats fed for 3 months with a diet in which 80% of the proteins were replaced by SCP. The UFA were about 3 times higher in the treated than in the control group, the maximum increase being for C17:1, followed by C17:0 and C15:0. The ratio of unsaturated to saturated fatty acids was similar for both groups. Since the presence of UFA in the membranes of the liver endoplasmic reticulum system could induce changes in their permeability, and therefore could affect the possibility of contact between substrates and enzymes, it was of interest to investigate the activity of liver microsomal enzymes obtained from controls, compared with rats treated for 3 months

Table 14.

Fatty acids profile of neutral (triglycerides and cholesterol) and polar (phospholipids) lipids in liver of rats

Fatty acids (a)	PERCENT OF TOTAL FATTY ACIDS			
	NEUTRAL		POLAR	
	controls	L 80	controls	L 80
14:0	1.2 ± 0.8	1.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.1
15:0	0.6 ± 0.1	1.9 ± 0.0	0.4 ± 0.0	1.6 ± 0.3
16:0	22.5 ± 0.4	16.3 ± 1.0	17.7 ± 1.7	19.1 ± 1.7
16:1	4.4 ± 0.3	4.4 ± 0.3	1.5 ± 0.0	1.5 ± 0.2
17:0	0.7 ± 0.1	2.2 ± 0.2	0.9 ± 0.2	3.2 ± 0.1
17:1	0.6 ± 0.1	3.5 ± 0.4	0.2 ± 0.0	1.2 ± 0,1
18:0	7.3 ± 0.8	12.8 ± 1.5	27.4 ± 2.3	24.0 ± 2.7
18:1	37.8 ± 1.3	30.7 ± 1.6	11.5 ± 1.0	13.2 ± 0.7
18:2	16.9 ± 0.4	15.6 ± 0.6	13.0 ± 0.9	11.7 ± 1.0
18:3	0.3 ± 0.0	1.0 ± 0.1	tr.	0.4 ± 0.0
20:1	0.6 ± 0.0	0.6 ± 0.2	0.7 ± 0.1	0.3 ± 0.0
20:2	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.2	0.5 ± 0.1
20:4	4.2 ± 0.6	6.9 ± 1.1	17.2 ± 1.7	19.0 ± 1.3
20:5 and 22:0	0.4 ± 0.1	0.2 ± 0.0	0.8 ± 0.0	0.4 ± 0.0
22:6	1.8 ± 0.3	1.4 ± 0.4	7.3 ± 1.5	3.4 ± 0.3
Uneven	1.9	7.6	1.5	6.0
Saturated	32.3	34.6	46.8	48.3
Monoenes	43.4	39.2	13.9	16.2
Polyenes	24.1	25.8	38.9	35.4

Rats were fed with L 80 for 2 months (treatment schedule as in Table 4).
Gaschromatographic analysis was performed as in Table 13.
a) carbon number of chain : number of double bonds.
Mean ± SE of 4 rats in each group.

with a diet containing 20, 50 or 80% of the proteins as SCP. Liver microsomal enzymes were prepared according to the technique described by Schenkman and Cinti (31) and incubated in presence of NADPH and 6-glucosephosphate dehydrogenase according to the method of Kato et al. (21).

The following reactions were studied: N-demethylation of aminopyrine (22); hydroxylation of aniline (12); 0-demethylation of p-nitroanisole (12) and nitroreduction of nitrazepam (1, 5). We also tested the activity of liver microsomal enzymes to conjugate p-nitrophenol with glucuronic acid according to the method described by Hollman and Tauster (17); Cytochrome P 450 and P_1 450 were determined according to the method of Omura and Sato (28). These studies revealed no difference between the control and the SCP treated groups, showing that the presence of UFA in the liver microsomal fraction did not affect the activity of liver microsomal enzymes for a variety of substrates (see Table 16). The epoxide synthetase and epoxide hydratase both enzymatic activities relevant to carcinogenesis were measured in SCP fed rats. The formation of epoxides is today considered

Table 15.

Fatty acid profile of total lipids of liver microsomes of rats

Fatty acids (a)	Percent of total fatty acids controls	L 80
12:0	tr.	tr.
14:0	0.4 ± 0.0	0.3 ± 0.0
15:0	0.3 ± 0.0	0.9 ± 0.1
15:1	tr.	tr.
16:0	20.4 ± 1.9	18.9 ± 1.1
16:1	1.7 ± 0.3	2.2 ± 0.4
17:0	0.8 ± 0.0	2.8 ± 0.3
17:1	0.4 ± 0.1	1.8 ± 0.1
18:0	23.1 ± 0.5	24.3 ± 1.8
18:1	15.1 ± 1.6	17.2 ± 0.4
18:2	14.7 ± 0.9	11.5 ± 0.9
20:4	22.0 ± 1.0	19.7 ± 2.5
22:0	0.5 ± 0.1	tr.
Uneven	1.5	5.5
Unsaturated	45.0	47.2
Monoenes	17.2	21.2
Polyenes	36.7	31.2

a) carbon number of chain: number of double bonds.
Mean ± SE of 4 rats for each group.
SCP fed for 3 months.

important in explaining the carcinogenicity of several polycyclic hydrocarbons (34). The method (2) permits separate measurement of epoxide synthetase, the enzyme responsible for the formation of styrene epoxide (20) and of epoxide hydratase, the enzyme responsible for inactivation of the epoxide into a glycol (27) (styrene dihydroxide). The results reported in Table 17 also list the effects of drugs known to affect these enzymatic activities. SCP has no effect on either enzyme, epoxide synthetase or epoxide hydratase.

Effect of SCP on fatty acid pattern in the brain of rats

The pattern of fatty acids in the brain was studied by feeding pregnant rats and, later on, the mothers and, finally, the weaned rats up to the age of 2 months with diets containing SCP (80% of the proteins). At the end of the treatment the rats were killed and their brains were analyzed for the fatty acid pattern. Table 18 shows that control rats already had low levels of C15:0, C15:1, and C17:1 in their brains, and that feeding SCP affected only slightly the UFA level. The ratio of unsaturated to saturated fatty acids was also not affected by treatment.

Table 16.

Microsomal enzyme activity in rats fed SCP for 3 months

PARAMETER	controls	L 20	L 50	L 80
a) Protein-9000 xg mg/g tissue	131 ± 4	121 ± 5	122 ± 5	126 ± 3
b) Protein-microsomes mg/g tissue	24.1 ± 2.5	23.8 ± 2.8	22.4 ± 4.2	27.4 ± 3.2
c) Aminazepam nmoles/mg/30'	1.56 ± 0.07	2.17 ± 0.17	2.17 ± 0.11	1.68 ± 0.1
d) p-Aminophenol nmoles/g/hr	1132 ± 45	1474 ± 102	1604 ± 130	1230 ± 115
e) p-nitrophenol nmoles/g/hr	1055 ± 91	990 ± 49	1132 ± 86	1318 ± 89
f) 4-aminoantipyrine nmoles/g/hr	735 ± 56	698 ± 45	922 ± 126	839 ± 57
g) UDPGA transferase nmoles/min/mg	2.45 ± 0.61	3.73 ± 0.47	2.34 ± 0.32	3.01 ± 0.49
h) Cytochrome P 450 nmoles/mg	1.82 ± 0.10	1.84 ± 0.24	1.81 ± 0.29	1.90 ± 0.12

Substrate disappearance was calculated per mg or g of protein concentration in 9000 xg preparation c, d, e, f, g; per mg of microsomal protein in h.

Table 17.

Epoxide synthetase and epoxide hydrase (mean ± SE) in liver microsomes of male and female rats

Treatment	Sex	Epoxide Synthetase nmol/min/mg protein	Epoxide Hydrase (microsomal)
Control	M	2.34 ± 0.13	4.23 ± 0.55
L 20	M	2.50 ± 0.12	5.16 ± 0.94
L 50	M	2.12 ± 0.11	5.68 ± 0.16
L 80	M	2.39 ± 0.10	5.34 ± 0.48
Phenobarbital	M	4.07 ± 0.78*	9.13 ± 0.94*
3-Methylcholantrene	M	3.61 ± 0.80	8.05 ± 1.37*
Control	F	1.19 ± 0.19	2.16 ± 0.29
L 20	F	1.52 ± 0.14	3.70 ± 0.38
L 50	F	1.85 ± 0.11	2.36 ± 0.22
L 80	F	1.72 ± 0.13	3.99 ± 0.48
Control	F	1.36 ± 0.24	3.49 ± 0.79
Norethisterone + Mestranol	F	2.13 ± 0.08	5.99 ± 0.28*
Phenobarbital	F	4.10 ± 0.30*	6.34 ± 0.38*
3-Methylcholantrene	F	1.88 ± 0.28	6.72 ± 1.28*

* $p < 0.05$
M = males; F = females.

Effect of SCP on fatty acid pattern and on platelet function

Platelets contain a relatively high level of phospholipids and this lipid class is considered important for their functional activities. Table 19 shows the effect of SCP feeding on the fatty acid pattern in platelets. Briefly, in rats, after 3 months feeding with SCP there is a slight increase in the UFA concentrations in total lipids. In view of these biochemical changes it was of interest to establish whether certain biochemical activities of platelets, involving membrane function, were affected by SCP treatment.

Platelets are known to concentrate serotonin (5HT) against a gradient with Km of 0.2-0.8 µM and a Vmax of 0.3-0.6 nmoles/10^8 platelets/min (39). Platelets from animals fed with SCP as 20% and 50% of the total protein showed no difference in respect to controls.

When the level of SCP was raised to 80% there was a reduction in both Km (from 0.84 to 0.38 µM) and Vmax (from 0.45 nmoles to 0.21 nmoles/10^8 platelets/min). However, a second experiment carried out with the same SCP concentration failed to confirm these results.

In other studies it was established that platelets obtained from rats fed SCP (80% of the proteins) for three months were similar to controls in their capacity to aggregate under the influence of ADP (1-2 µM). The method used was that of Born and Cross, as previously described (8). Table 20 shows relevant hematologic parameters together with the number of

Table 18.

Fatty acids profile of total lipids of brain of rats fed SCP for 2 months

Fatty acids (a)	PERCENT OF TOTAL FATTY ACIDS control	L 80
14:0	0.4 ± 0.0	0.3 ± 0.0
14:1	tr.	tr.
15:1	0.2 ± 0.1	0.3 ± 0.1
16:0	20.0 ± 1.5	19.2 ± 1.0
16:1	0.4 ± 0.0	0.5 ± 0.1
17:0	0.2 ± 0.0	0.5 ± 0.1
17:1	tr.	0.3 ± 0.0
18:0	22.0 ± 0.8	19.3 ± 1.9
18:1	23.0 ± 0.8	21.2 ± 1.7
18:2	0.9 ± 0.1	0.8 ± 0.0
18:3	tr.	0.2 ± 0.0
20:0	0.7 ± 0.1	0.6 ± 0.0
20:1	2.4 ± 0.5	2.5 ± 0.1
20:4	10.0 ± 0.4	13.0 ± 1.6
20:5 - 22:0	0.7 ± 0.1	0.6 ± 0.0
22:6 and others	18.7 ± 0.9	20.1 ± 1.2
Uneven	0.4	1.1
Saturated	43.5	40.2
Monoenes	25.8	24.5
Polyenes	30.3	34.6

Treatment schedule as in Table 4.
(a) carbon number of chain: number of double bonds.
Mean ± SE of 4 rats per group.

platelets and in addition the bleeding time and platelet factor 3 activity according to the methods of Stella et al. (35) and Hardisty and Hutton (15). Both tests were selected to give further information on platelet function. In these tests too, SCP treated animals were comparable to the controls.

Effect on other hematological parameters

Other hematological parameters were evaluated in rats treated with SCP. The fibrinogen content was measured according to Donati et al. (9); the fibrinolytic potential (11); fibrinogen degradation products (16); both prothrombin time and partial thromboplastin time (29); erythrocyte osmotic fragility tested according to Dacie and Lewis (7); and macrophage function tested according to Benaceraff et al. (3). No difference between SCP and control animals could be detected.

Conclusions

The data presented are in good agreement with other results available in the literature. The adipose tissue triglyceride composition reflects that of the fatty acids present in the diet (19). It is therefore no surprise that adipose tissue of several animal species,

Table 19.

Fatty acids profile of total lipids of platelets in rats fed SCP for 3 months

Fatty acids (a)	Percent of total fatty acids controls	L 80
8:0	0.6	0.9
10:0	0.7	1.0
12:0	2.1	2.5
14:0	5.1	6.1
15:0	1.2	2.6
16:0	25.8	30.5
16:1	1.9	3.1
17:0	0.8	2.1
17:1	2.8	3.0
18:0	16.1	19.4
18:1	3.9	3.4
18:2	3.6	2.5
20:0	0.3	0.3
20:4	4.1	2.3
20:5 - 22:3	21.7	9.8
Uneven	4.8	7.7
Saturated	52.8	65.4
Monoenes	8.6	9.5
Polyenes	29.4	14.6

(a) Carbon number of chain: number of double bonds.
Figures are pool of 4 rats.

including mice, rats, chicken and monkeys, fed with a diet containing SCP rich in uneven fatty acids (UFA) also becomes rich in such fatty acids, mostly C15 and C17. It should be underlined that the adipose tissue of animals fed with control diets also shows measurable amounts of UFA. Animals fed with increasing amounts of SCP show increasing concentrations of UFA in their adipose tissue. However, the percentage of UFA in adipose tissue rapidly reaches a plateau and increases no further with the duration of SCP administration (from 2 to 15 months).

These data are consistent with the hypothesis that mammals are equipped with the biochemical mechanisms to metabolize UFA (3). This hypothesis is confirmed by experiments in which animals with increased levels of UFA when fed normal diets showed a reduction in the UFA of the adipose tissue which returned to control values within one month. The increase of UFA in animals fed SCP is not limited to the adipose tissue, but is also noted in the heart, liver and platelets, but not to the same extent in the brain. On a percentage basis the UFA are higher in the triglycerides than in the phospholipids for the liver.

The lipid pattern in various rat tissues was not substantially altered by administration of SCP. Only at the highest doses (replacement of 80% of dietary proteins) did SCP

Table 20.

Hematologic parameters in rats fed SCP for 3 months

	controls	L 80
Platelets $10^3/\mu l$	903 ± 57	961 ± 24
Red cells $10^3/\mu l$	8084 ± 296	7585 ± 129
Leucocytes/μl	18100 ± 1837	14220 ± 1687
Platelet factor 3 (sec.)	45.6 ± 3.3	46.2 ± 2.8
Red cell fragility (% NaCl)	0.38 ± 0.01	0.36 ± 0.01
^{14}C-5HT uptake Km (μmoles)	0.27 ± 0.03	0.24 ± 0.03
^{14}C-5HT uptake Vmax nmoles/10^8 plat/min	0.24 ± 0.01	0.22 ± 0.02
Bleeding time (sec.)	87 ± 7	102 ± 12

reduce the level of adrenal cholesterol and liver triglycerides. Because of the reduction of adrenal cholesterol, the reaction of rats on exposure to cold was studied. No difference could be found between controls and the treated groups as regards increases of plasma corticosterone, plasma FFA and liver triglycerides. Only with the highest dose of SCP was there less accumulation of liver triglycerides in treated animals than in controls following exposure to cold. The results indicated in general that the mobilization of lipids from adipose tissue was not influenced by the presence of UFA. This conclusion was supported by the finding that animals fed with SCP behave in the normal way when submitted to exercise (swimming and rotarod test) under experimental conditions requiring adequate availability and utilization of energy giving substrates.

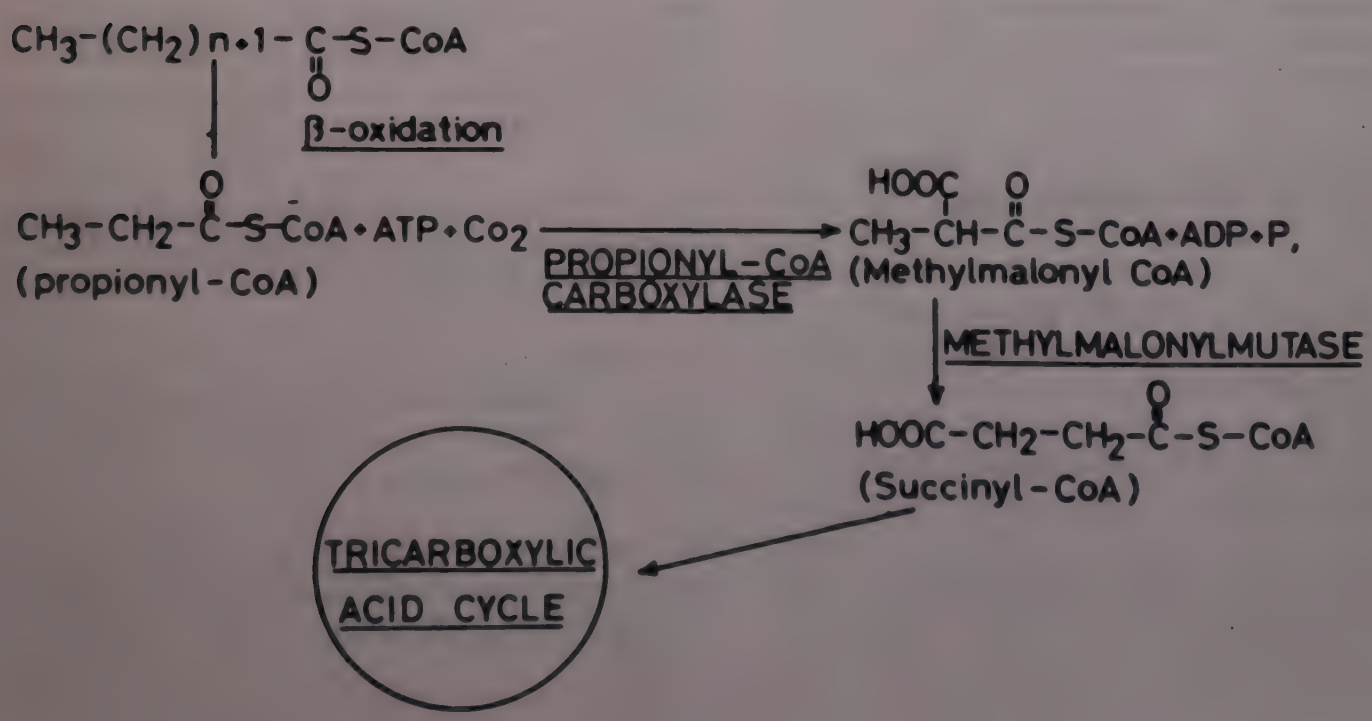
The presence of UFA in the heart led us to investigate cardiac function; SCP treated and control rats showed a similar ECG pattern as well as similar responses to pharmacological agents capable of increasing heart rate.

Accumulation of UFA in the liver did not affect the endoplasmic reticulum system function as far as the microsomal enzymes are concerned. The levels of liver cytochrome P450 and of microsomal proteins were also not affected by SCP treatment. Similarly a number of substrates which are metabolized by the liver microsomal enzymes behave in the same way when tested in vitro with such enzymes obtained either from control or SCP treated animals. The metabolic reactions investigated include aniline hydroxylase, aminopyrine-N-demethylase, p. nitroanisol-O-demethylase, p. nitrophenol conjugation, nitrazepam nitroreductase, styrene epoxide synthetase and hydratase. The presence of UFA in platelets suggested a number of studies all of which failed to show any substantial difference between controls and SCP treated rats; these included number of platelets, platelet aggregation, serotonin uptake, bleeding time and other hemostatic parameters.

The findings are consistent with the hypothesis that the presence of UFA in the body at limited concentrations, not exceeding 10% of total fatty acids, is compatible with normal physiological functions since UFA can be metabolized and utilized.

Figure 1.

Principal metabolic pathways of uneven fatty acids oxidation



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SAFETY EVALUATION STUDIES WITH SCP

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Introduction

In the initial phase of the development of processes for industrial production of single cell proteins, it was assumed that the products as such would be used as constituents in the diet of humans. For this application, careful evaluation of the nutritive quality and of the safety of the products was considered essential. It was clear that the safety evaluation could best be conducted by the usual procedures for the examination of the toxicological properties of additives and contaminants in human foods. Detailed recommendations for testing procedures were presented in the PAG guidelines 6 and 7.

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It later appeared, however, that SCP would be used primarily as feed for animals, rather than as food for humans. One might expect ready acceptance of the application of SCP to animal feeds, because new feed compounds are generally used on the basis of their nutrient contents, without prior safety testing. However, instead of simplifying the evaluation, the introduction of SCP for feeding animals gave rise to various extensions of the proposed testing program. In addition to the classical toxicity studies in laboratory animals, feeding experiments in the target species, and toxicity studies with edible products derived from these species, are now being advocated. This would imply a more extensive testing program than ever required for any additive or contaminant in the human diet.

The issue is considerably simplified by making a clear distinction between nutritive evaluation and toxicity testing. Only the latter aspect is considered in this paper.

Pitfalls in safety studies

Since safety studies are intended to demonstrate, if possible, harmful properties, the products to be examined are fed at a dietary level as high as possible without inducing nutritional imbalances. Lower levels are examined to discover a possible dose-effect relationship and to establish a no-toxic effect level. Care is taken to equalize, as far as possible, the dietary contents of nutrients and roughage, because both excesses and deficiencies may induce deleterious effects which wrongly arouse doubt about the safety of the products examined.

Excess of dietary phosphorus: SCP products are usually high in phosphorus and low in calcium. A very high level of SCP in the diet may, therefore, result in a relative excess of phosphorus. Diets with a Ca:P-ratio above one, may induce alterations in the kidneys characterized by deposits of calcareous material, mainly in female rats. If the dietary level of phosphorus is not too high, the renal condition can be prevented by supplementing the diets with a source of well-soluble calcium, e.g., $CaCl_2$.

Deficiencies of copper and selenium: Certain SCP's are low in copper or selenium. The dietary incorporation of these products at the expense of conventional protein concentrates may result in deficiencies, which rapidly induce pathological changes in the test animals. In the case of copper deficiency, anemia develops and the sudden death of seemingly healthy rats of normal body weight may occur. Selenium deficiency results in growth depression and pathological changes in liver.

Deficiency of potassium: The potassium level of normal feed compounds is sufficiently high to cover the needs of animals fed conventional diets without a potassium supplement. When conducting short-term feeding tests in rats with experimental yeast samples, the animals showed growth depression, increased kidney weight, and pathological changes in the renal tubules. The conclusion that the samples were toxic turned out to be premature. For the purpose of reducing the contents of nucleic acids, the yeasts had been subjected to alkaline washing which removed the major part of the potassium. Because semi-purified diets were used, the incorporation of the yeasts at the expense of soybean oil meal resulted in low potassium levels. When the same yeast diets were fed with a supplement of K_2SO_4, no abnormalities occurred (Gatumel and Shacklady, 1975).

Texture of the diets: In addition to the contents of nutrients, the physical structure of the diets may be a reason for suspicion with respect to the safety of SCP. It is known that dusty diets tend to depress food intake in chickens and to induce clogging of food in the corners of the beak (Waldroup and Payne, 1974). The feeding of a diet containing a high level of a dusty SCP to Japanese quail induced poor growth and mortality. Both abnormalities were obviated when the particle size was improved by pelletizing and regrinding the diet.

Results of safety-evaluation studies

In the toxicological literature only a few papers have appeared which deal with the safety-evaluation of SCP.

Studies conducted with a yeast and a bacterial biomass, both grown on pure hydrocarbons, have been published by Swedish workers (Agren et al., 1974). The products were fed for thirteen weeks to groups of six male and six female rats, either after adding them to stock diet (at levels providing fifteen or twenty-five per cent additional protein), or as the only source of protein (at levels providing twenty per cent crude protein). Methionine was not supplemented, and it was not mentioned whether the diets were balanced with respect to calcium and phosphorus.

The rats fed the highest level of the yeast or bacteria with stock diet showed kidney enlargement. Since the protein level of these diets was as high as forty per cent, this abnormality might be due to excessive protein intake. Diets containing the products as the only source of protein at levels providing twenty per cent crude protein induced growth depression and nephrocalcinosis. In addition, high mortality, testicular atrophy, and hematological changes occurred in rats fed twenty per cent bacterial protein. These effects were attributed to some toxic factor in the bacteria biomass. Since a high incidence of nephrocalcinosis was observed, a Ca:P imbalance might also be responsible for the poor results obtained in this study.

Recently, Canadian workers published results of toxicity studies with a fungus grown on natural gas. The biomass was fed to groups of six rats at twenty and forty per cent in a semi-purified diet, at the expense of casein, for five months. The fungus diets caused growth depression, decreased leucocyte counts, and increased kidney weights. No pathological changes were encountered upon microscopy of the major organs (Volesky et al., 1975).

The yeast produced by BP from pure n-alkanes has been examined in the Central Institute for Nutrition and Food Research by the usual toxicological studies for additives in foods. The rat was used as the main test species, but long-term studies also have been conducted in mice and Japanese quail. The diets contained ten, twenty, or thirty per cent yeast, incorporated mainly at the expense of soybean oil meal in a basal diet containing twenty-seven per cent crude protein. The Institute's stock diet with twenty-three per cent crude protein served as a second control. The diet with the highest level of yeast contained nearly one per cent odd-numbered fatty acids and 1500 ppm residual paraffins.

The design of the sub-chronic and chronic studies is shown in Table 1.

Table 1.

Design of Subchronic and Chronic Studies

Observations	Studies in rats			Carcinogenicity study in mice
	Subchronic	Chronic	Carcinogenicity	
Period	90 days	2 yr	2 yr	1 1/2 yr
Animals per group	20	50 - 60	100	200
Health	daily	daily	daily	daily
Body weight	weekly	monthly	monthly	monthly
Food consumption	at intervals	at intervals	-	
Hematology	wk 12	at intervals	-	wk 52, 78
Blood biochemistry	wk 12	at intervals	-	
Urine composition	wk 12	at intervals	-	wk 78
Kidney function	wk 12	at intervals	-	
Autopsy	wk 13	wk 104	wk 104	wk 80
Organs weighed	9	9	4 - 5	6 - 7
Gross pathology	all rats	all rats	all rats	all mice
Histopathology	all rats	all rats	all rats	40/group and tumors

Two multigeneration studies were conducted, one with rats, the other with the Japanese quail. Separate studies in rats have been conducted for possible teratogenic or mutagenic properties. Detailed examination of the skeleton and soft tissue of the fetuses were made to discover birth defects, while the dominant lethal assay in male rats was used to detect changes in genetic properties.

The results of the two-year rat study showed no adverse effects on mortality, growth rate, hematology, urine composition or kidney function. The activities of serum enzymes were relatively high but within the normal range. There were no changes in the incidence or severity of histopathological changes, nor in the type and incidence of tumors. The multigeneration study in rats revealed no effects on fertility, the number of young, or the growth and mortality of the young during lactation (deGroot et al., 1975). The carcinogenicity studies in rats and mice, and the teratogenicity, and mutagenicity studies in rats were also negative. In the quail study, normal egg production and fertility was maintained on the thirty per cent yeast diet in successive generations. After completion of the usual procedure for reproduction studies, selected rats and quail of each group were continued on their diets for rearing further generations. At present, the nineteenth generation of rats and the twenty-ninth generation of quail have been reared. So far, no indications of decreasing performance have been noticed.

It can be concluded from these studies that the feeding of the yeast examined at a very high dietary level to several species of laboratory animals for the major part of their lifetime and for many successive generations, gave no evidence of a deleterious effect.

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TOXICOLOGICAL STUDIES WITH "PRUTEEN"[1]

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Introduction

"PRUTEEN" consists of the dead dried cells of a non-pathogenic bacterial organism, Methylomonas methylotropha. It is an organism which will grow only on methanol. Methanol is added to the fermenter liquor at a concentration of five per cent, and is immediately metabolized by the organism. Methanol cannot be detected in the fermenter liquor, as the biomass material is removed from the fermenter, or in the final dried product, at a level of eight ppm, which is the limit of chemical detection. Ammonia is used as the nitrogen source. The product is produced by continuous fermentation and drying. No antibiotics or other growth promoters are used, and hence the product is essentially a cellular product, together with soya bean oil, which is present due to the need to control frothing on the surface of the fermenter.

The industrially produced SCP's, namely the yeasts, fungi, and bacteria, are different in their properties. These SCP's may be grown on a range of carbon substrates, from gas oil, through refined hydrocarbons, to methane, alcohols, and waste carbohydrates. The apparent toxicological risks, therefore, differ with each SCP process. The Federal Drug Authority of the United States has accepted this philosophy and allows Torula yeast grown on chemically produced pure alcohol (ethanol) as a human food; the degree of toxicological testing to support this product has been small in comparison to the yeast grown on hydrocarbons for animal feeds. The above statement is not meant to query the relative degree of testing which is needed for an SCP, but to point out that not all SCP's are the same, and that any guidelines on testing SCP's are intended for the mutual guide of both the scientist setting up tests to determine the degree of toxicological risk, and the external evaluator of the final information. This is a point well understood by scientists, but less easily comprehended by non-scientifically trained personnel, who may regard the guideline as a compulsory shopping list that has to be followed.

Toxicological testing of "PRUTEEN"

As "PRUTEEN" is intended as an animal feedingstuff, it should be demonstrated as being safe and nutritious for those animals that will eat it. It should be shown that it will allow them to grow, reproduce, and produce food fit for human consumption. This information is not only needed by governmental authorities, but also by any self-respecting industrial organization for its own information to insure that there is no risk to the public. These tests are best carried out on the farm animals that will be the direct recipient of the product, as they produce the meat that humans consume.

This toxicological testing on the farm animal, the so-called target species, is not so straightforward as it may sound. Within the science of toxicology, there is considerably less understanding of what is meant by the normal situation in histopathological, hematological, etc., terms for the farm animal, in comparison with the rat. Similarly there is a poor understanding as to how variation in basic dietary nutrients affects the normal parameters studied in the rat. The net result is that in the evaluation of a feeding material there has to be a compromise between the way in which a nutritionist and toxicologist would set up these experiments. Furthermore, because of the uncertainties which may exist in understanding the target species experiments, studies on the conventional laboratory animal are also needed. This is best illustrated by the type of work which

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1. "PRUTEEN" is the registered trade mark for the SCP produced by ICI.

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has been carried out on "PRUTEEN". A large amount of work has been done, and it is only possible to quickly survey the type of test and summarize the results in a short paper.

Experiments with "PRUTEEN" in laboratory animals

1. Pathogenicity: The process organism, viable Methylomonas methylotropha, has been tested by conventional pathogenicity tests in mice. The test dose was 1 ml containing 10^9 organisms/ml. It is not pathogenic, and bears no relationship to known pathogenic species of bacteria.

2. Sub-chronic and chronic studies with rats: "PRUTEEN" has been tested in combination with either natural or purified diets in ninety-day studies using graded levels of SCP with fishmeal or casein as a control protein. In the chronic study a basal low protein diet containing eight per cent protein was formulated and a further eight, sixteen, or twenty-four per cent crude protein was added from either casein or "PRUTEEN". The maximum "PRUTEEN" incorporation rates are expressed in Table 1. No changes were observed in any of the experiments. These high protein levels are considerably in excess of the protein requirements of the animal, and casein was specifically included as a control material that is conventionally accepted as being safe. The only major unusual feature has been an increase in kidney size, which was connected with protein rather than "PRUTEEN", together with some histological changes in the kidney.

Table 1.

	Max Diet Protein %	Control Protein	Max "PRUTEEN" conc %
Rat 90 day	27	Fishmeal or casein	13
Rat 90 day	20	Casein	26
Rat 2 year	32	Casein	30
Dog 6 week	44	Casein	60

Short term experiments with dogs in which sixty per cent "PRUTEEN" was incorporated in the diet revealed no problems. This dietary "PRUTEEN" level was equivalent to a daily "PRUTEEN" intake of 12g/kg body weight per day. This should be compared with the response to single doses of a chemical, in which values of 5g/kg are considered to be harmless if no positive effect is obtained.

3. Reproductive tests with rodents: reproduction as such, and the allied toxicological evaluations associated with the production function were studied in rats and mice. There have been no differences in the reproductive rate, or in the number of abnormal fetuses between the control and the test groups. Specific teratological experiments have also been negative. A dominant lethal study to seek mutagenic effects has been made on mice with negative effects; the control material ethylmethanesulphonate was strongly positive in producing this effect.

The overall conclusion reached on these classical toxicological tests is that within the limits of the science as it currently stands, "PRUTEEN" is not having a harmful effect. There is no evidence of carcinogenesis, and reproductive factors are not affected in any way whatsoever. Hematological characters are not changed. The same applies to the blood biochemistry, with the exception of those parameters which may be expected to change. Thus, blood urea is elevated, due to the need to excrete excess protein in high protein-containing diets. There is a constant plasma uric acid and an increased blood and urinary allantoin, indicating that the purines from the nucleic acids that are present in "PRUTEEN", at a level of fourteen per cent, are being degraded to the final catabolic end product and are being excreted.

Toxicological testing with the target species

Compared with the conventional toxicological tests, the use of target species presents a dilemma as to how long these tests should last. Life-time studies with the pig (say fourteen years) and the fowl (say seven years) are clearly not practical, and are totally impossible to interpret. It is because of this that it is necessary to use the rat as an experimental model. ICI has solved the problem by allowing the test to proceed for the normal commercial lifespan of the animal.

The amount of "PRUTEEN" which should be

added to the diet also presents problems in that excessive levels create dietary imbalance, which affects growth. For the short term studies, this problem has been met by either feeding substantial amounts of "PRUTEEN", vastly in excess of the protein requirements of the animal, or in separate experiments, by feeding more limited amounts of "PRUTEEN", up to the normal nutritional protein requirements. For the longer term studies, the maximum dietary protein level defines the maximum "PRUTEEN" dose. In all studies, a dose/response experimental design was used.

Experiments with "PRUTEEN" on the target species

The main target species are poultry, pigs, calves, fish and domestic animals. This paper will only discuss experiments with the first three species.

Poultry: Acute, sub-chronic, and reproduction tests, including fertility and hatchability of the egg, have been made with this species. The type of test, inclusion rate of "PRUTEEN", and results are summarized in Table 2. The twenty-one day acute studies were used as preliminary screen for the development of the process, while the longer fifty-six day broiler studies help to evaluate the problems, if any, from feeding "PRUTEEN". Similar tests have been made with laying and breeding hens.

Table 2.

Toxicity Test
Target Species: Fowl

Study	Max Diet Protein %	Control Protein	Max "PRUTEEN" conc %
Broiler, 56 day	27	Fish/soya	25
Broiler, 56 day	20	Fish/soya	12
Broiler, 21 day	20	Purified soya	27
Layer, 20 months	16	Fish/soya	10
Layer, hatchability	16	Fish/soya	10

The results of these tests are very similar to those on the rat. There have been no pathological, histopathological, hematological, or biochemical differences between test and control groups. The reproductive studies show that hens lay well on diets containing "PRUTEEN", and that there are no differences in fertility or hatchability. These tests have been quite large. A typical broiler or laying hen test will involve 1,500-1,600 animals, while the breeding test utilizes about 500 hens in a multi-generation experimental design.

Pigs: Sub-chronic and reproduction tests have also been made with the pig. Here again the results show a similar pattern. The conventional toxicological data shows no differences from the control. Young female pigs killed during their first pregnancy show no teratological abnormalities in the fetuses they carry. Reproductive performance is the same as the control (Table 3).

Table 3.

Reproduction Performance

	Control	10% "PRUTEEN"
No. of litters	99	104
At Birth		
No. born/litter	10.51	10.66
No. born alive/litter	9.96	9.86
Total litter weight (kg)	12.78	13.14
At 3 weeks		
No. alive	8.31	8.46
Total litter weight (kg)	44.06	44.40

Veal Calves: Only sub-chronic tests have been made on veal calves; it is assumed that these animals will not reproduce. A similar pattern of tests as to the other target species have been undertaken. The most extreme "PRUTEEN" level has been twenty per cent but most tests have been evaluating a five and ten per cent incorporation level. Again, the toxicological results have been similar to the controls.

Conclusion on toxicology

The rat, dog, pig, veal calf, and fowl all behave in the same manner. There is no evidence of tumor growth, carcinogenesis, or any other abnormality that cannot be ascribed to the use of excess protein as such. "PRUTEEN" behaves in the same

manner as casein, soya bean or fish. Excess protein causes kidney enlargement and some degeneration of the proximal tubules in casein-and "PRUTEEN"-containing diets. Changes are also seen in blood urea, in control and experimental diets. In the "PRUTEEN"-fed animals there was no evidence for elevated blood uric acid, but conversely, blood allantoin was elevated. This, coupled with the presence of allantoin in the urine, and nutritional studies demonstrating complete digestion of the nucleic acid component, clearly suggests that the unusually high nucleic acid component of "PRUTEEN" does not give cause for concern. The above changes are not observed at normal dietary inclusion levels, and hence it can be concluded that "PRUTEEN" is a harmless ingredient for the purpose of feeding to animals.

Safety of meat

Apart from demonstrating the fact that "PRUTEEN" is safe for animal consumption, it is desirable to demonstrate the degree of risk which the human population will face eating "PRUTEEN"-fed animals. Attention has to be focused on those components which accumulate within the body, namely heavy metals and fatty acids, and these features have been routinely tested in experiments.

The heavy metal content of "PRUTEEN" is exceedingly low, and no accumulation of As, Hg, Cd and Pb has been observed in any species.

The fatty acid profile of "PRUTEEN" shows that the material contains palmitoleic acid in fairly substantial quantities. The fatty acid profile is given in Table 4 and is compared with other naturally occurring products. Odd chain fatty acids are not found in "PRUTEEN".

The effect on the tissues of various animals fed substantial quantities of "PRUTEEN" has been studied, and the results are given in Table 5. Clearly, there is a marginal increase in the palmitoleic acid content, but these levels are not large and no harmful characteristic has been ascribed to this acid. The variability in the fatty acid composition induced by "PRUTEEN" feeding is little greater than that produced by feeding products such as soya and fish.

As a result of this work, where the extreme situation has been tested, we are not aware of any reason why "PRUTEEN"-fed meat should not form part of the normal diet of the human population.

General conclusions

"PRUTEEN" has been extensively tested in both laboratory and target farm animal species. The only changes which have been detected are those which may be expected from excess protein feeding, or from the changes in animal biochemical patterns associated with the need to degrade nucleic acid. On the basis of the evidence available, "PRUTEEN" appears to be a harmless product suitable for animal feeding. Similarly, the meat derived from these feeding studies does not accumulate toxic heavy metals and there are only marginal changes in the fatty acid profile of the adipose tissues, resulting in a minor accumulation of palmitoleic acid.

Table 4.

Fatty Acid Composition

Fatty Acid	"PRUTEEN"	Lard	Butter	Cod Liver Oil	Soya Oil
14:0	1.4	1.3	11.1	5.8	0.1
16:0	35.3	28.3	29.0	8.4	9.8
18:0	2.5	11.9	9.2	0.6	2.4
16:1	35.0	2.7	4.6	20.0	0.4
18:1	9.9	47.5	26.7	29.1	28.9
18:2	8.6	6	3.6		50.7

Table 5.

"PRUTEEN"

Effect upon Carcass Composition: Fatty Acids

	Pig Cutaneous Fat % "PRUTEEN"			Egg Yolk % "PRUTEEN"			Fowl Fat % "PRUTEEN"		
	0	10	30	0 Soya	0 Fish	10	0 Soya	0 Fish	10
14:0	1.3	1.2	1.2	0.3	0.3	0.4	0.5	0.7	0.7
16:0	23.4	24.2	24.9	25.6	25.6	24.2	18.9	20.7	20.4
18:0	14.2	15.5	14.9	8.8	9.4	9.1	4.8	5.8	5.5
16:1	2.9	4.2	7.1	2.4	2.7	3.6	3.4	4.2	5.9
18:1	41.9	40.3	39.5	44.4	48.1	45.4	45.4	49.6	44.9
18:2	13.0	11.7	10.3	16.4	11.1	13.4	25.7	17.3	21.1

SUMMARY OF TECHNOLOGICAL DEVELOPMENTS OF HYDROCARBON-GROWN YEAST

B. M. Laine*

British Petroleum has developed two processes using either raw gas oil or pure normal paraffins as feedstock. In the gas oil route, raw gas oil boiling at 300-380° C range is fed continuously with an aqueous medium containing all the necessary nutrients to a reactor where a strain of yeast - a Candida tropicalis - is growing. The yeast selectively consumes the normal paraffin fraction, as a source of carbon and hydrogen, for metabolism and growth. Fermentation broth is continuously drawn from the fermenter and the yeast biomass produced is separated from the non-consumed gas oil. After fermentation and separation, the biomass is subjected to a very extensive solvent purification process. This ensures removal of any traces of hydrocarbons that might remain in the biomass.

In the pure normal paraffin route, paraffins of high purity (over 99%) with carbon numbers ranging from C_{10} - C_{23} are used. The purity of the animal feedstock is such that it passes the various U.S. and Western European regulations for the use of hydrocarbons in feed; the most important of these being the U.S. Food and Drug Administration test 121.1146, which forms the basis of the WHO specification. In practical terms, passing the FDA test means that if there are traces of polycyclic aromatics (e.g. benzpyrene) present in the normal paraffins, they are present below the parts per billion level.

Normal paraffins are again fed continuously with an aqueous medium. As the fermentation is aseptic, each feed stream, except the yeast inoculum, must be sterilized prior to entering the fermenter. In the process of growth, the yeast used - a Candida lipolytica - consumes practically all normal paraffins requiring only separation of the yeast biomass produced from the spent aqueous medium. This is done by centrifugation, followed by spray drying.

The 16,000 t/y unit using the gas oil route, built at Lavéra, (France) has been shut down. The possibility of switching the plant to a pure normal paraffin feed is being investigated. This does not mean that BP has lost confidence in the gas oil route, which can produce an SCP of extremely high nutritional quality, as years of experience in the market place have proved.

*Assistant General Manager, BP Proteins Limited.

Following the 1973 Yom Kippur war, oil prices have increased considerably and the minimum economic plant size for SCP production has risen accordingly. Operation of the Lavéra plant had shown that the solvent extraction unit is a bottleneck to production, while there is effectively a fermentation capacity in the existing unit of up to 30,000 t/y. Therefore, it has been decided to make use of this extra capacity - hence the current investigation into the use of normal paraffins which would by-pass the extraction unit.

The normal paraffin substrate has been used for a 4,000 t/y design capacity demonstration unit at Grangemouth, Scotland. Its operation since 1971 has led BP to develop in great detail and, it is hoped with great reliability, BP's normal paraffin process. This is the process which will be used in BP's joint venture with the state chemical enterprise, ANIC, of Italy, where BP has completed construction of a 100,000 t/y plant at Sarroch in Sardinia. BP has also two 100,000 t/y projects in the making: one in Venezuela, jointly with the Venezuelan Government, for completion in 1978, and one in Saudi Arabia.

Most of the other developments in Europe have stemmed from work on normal paraffin processes initiated in Japan by Kanegafuchi Chemical Industry Co. Ltd., and Dai Nippon Ink and Chemicals Inc. Kanegafuchi has licensed its process to Liquichimica of Italy, which has completed construction of a 100,000 t/y plant at Saline di Montebello in Calabria, Southern Italy.

Judged from the literature available, this process differs from the BP process in a number of ways. First, the Kanegafuchi/Liquichimica process is non-sterile, and is based on continuous cultivation of a mixed yeast population which can be stabilized by an adequate selection of operating conditions. The principal organism used is apparently a Candida novellus, identified by some as Candida tropicalis, and by others, as Candida maltesa. Second, the fermentation takes place in air lift reactors instead of stirred baffled reactors at 37°C instead of at 30°C in the BP process.

Dai Nippon has developed a process based on a species of pichia yeast grown in an aseptic manner. Cultivation takes place in two fermenters, the first one being used for cell propagation, and the second, for consumption of residual normal paraffins. The company has begun construction of a 60,000 t/y plant in partnership with the Rumanian Government, for completion in 1977.

This is the situation in Europe at the present time. Very large operations are becoming a reality, and the moment is arriving when it can be judged whether the technological development has been properly guided, and whether scaling up has been carefully and correctly applied. If this is so, the road will be clear for further large production.

SINGLE CELL PROTEIN FROM METHANOL

G. H. Pace*

A number of organizations in Japan, Scandinavia and Germany are actively investigating processes using methanol as feedstock, for protein production using either bacteria or yeasts. Work using ethanol as a feedstock is also under way in the U.S.A., Italy, and Czechoslovakia. Extensive work has also been carried out on methane/bacteria systems. Thus there is a variety of routes of investigation and the use of methanol and ethanol with similar properties can give rise to similar processing problems. The Imperial Chemical Industries Limited (ICI) process for production of single cell protein uses methanol as the feedstock. The bacterial organism is Methylomonas methylotropha.

Around 1968, ICI began looking into the possibility of manufacturing single cell protein. After careful consideration of a wide variety of factors, it was decided to adopt the methanol/bacteria route. Many organisms

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were screened and the one chosen most closely matched the desired set of characteristics. The next big question was how to scale up most reliably the process to commercial size from small laboratory fermenters of only five liter capacity.

Fundamental technical issues requiring solution were seen to be: 1. Achieving high volumetric transfer rates of oxygen at low power inputs; 2. Removal of the heat of fermentation at low temperatures; 3. Continuous operation of the fermentation under completely sterile conditions; 4. Separation of the organism from the resulting culture; 5. Producing a final product of regular composition without subsequent contamination in the separation and drying processes; and 6. The design of an inherently simple plant using the modern single stream philosophy.

There are formidable difficulties in scaling up a laboratory fermenter to a full size plant. The rapid mixing times achieved in the laboratory scale, so necessary for this particular culture system, are not readily achievable in large stirred vessels. A decision in principle was taken to tackle the problem by eliminating the need for externally driven stirrers, to assist sterility by in-line cooling within the fermenter system and to make it possible to construct very large single fermenters. The novel pressure cycle fermenter was therefore developed.

The fermenter is made up of two tall vertical columns connected at the top and bottom. The large column is the riser into which air and fresh medium are introduced at the base. The two-phase mixture flows upwards, and the voidage in this riser being higher than in the downcomer, the resulting hydrostatic head difference provides a driving force for a rapid circulation. Excellent oxygen transfer is achieved because of the high hydrostatic head and the level of turbulence promotes a small bubble size and good transverse mixing. The presence of the inert nitrogen content of the air acts as a stripping gas to prevent accumulation of CO_2 in the liquid. Some fifty per cent of the influent oxygen dissolves. In the upper horizontal section the spent air is disengaged before the liquor passes down the downcomer. The downcomer is of smaller diameter than the riser and high velocities are achieved. The simple heat exchanger set into the downcomer enables heat to be removed under ideal conditions before the liquid is returned to the base of the riser.

Maintenance of sterility in such a vessel is assisted by the fact that the only connections are the inlet and exit media and air streams; the necessary stirring work is imparted by the expansion of the air within the device. While the route is properly described as being methanol/bacteria, it should be noted that the fermenter liquor is a suspension of the organism in water to which nutrients are added in the required concentration. Methanol never exceeds parts per million concentration.

The small size of the bacteria make removal of the organism from the fermenter culture difficult. Therefore, a separation stage has been devised which enables the production of large flocs without any need for the addition of undesirable flocculating agents to the final product. This step has been developed to the point where further concentration by centrifuges is uneconomical. The step greatly simplifies the process: it removes a state of mechanical complexity and reduces power consumption. Conventional drying equipment is used to dry the separated product to a moisture content suitable for handling and storage. The water separated from the flocs is returned to the fermenter to reduce the water requirement for the process and to enable the small quantity of useful material in this stream to be recovered.

A pilot plant of 1,000 t.p.a., constructed to test these concepts and to prove that continuous sterile operation was possible, has now been operating for three years. The plant is a tall structure of about 30m. The principles of the pressure cycle fermenter and the harvesting process have now been proven, and runs of over three months continuous sterile operation have been achieved. The plant has produced quantities of material to enable the extensive testing of its nutritional and toxicological properties. The product is also being used in commercial diets in animals, to illustrate and determine the desirable features it contributes to those diets and to the growth and health of these animals.

The detailed design of the commercial plant is now at an advanced stage. The basic flow diagram follows closely that

used in the pilot plant. The lessons learnt on the pilot plant have been incorporated into this design, in particular, sterilizing the inputs; keeping the fermenter sterile; dealing with the fair-sized heat load (the fermenter operates at 40°C); the separation process; recycling the liquor and the conventional drying units; and, as in the case of all industrial plants these days, taking particular care of liquid and gaseous effluents. To eliminate operator error in this large plant, the operation of a fermenter is to be supervised by a computer.

The subsequent processing of the protein takes place under hygienic conditions appropriate to a food processing plant, in order to minimize subsequent contamination. This operation is made easier by the simple separation and drying techniques employed which do not require the use of centrifuges. The final products have been made physically suitable for their role in the animal feed into which they will be incorporated.

Prominent features of the plant are to be the large cooling tower to dissipate the heat of fermentation, and the tall fermenter. The plant has now been simplified to get the riser and downcomer within a single shell, a design suited to sterile operation. In every aspect, particular care has been taken to ensure the highest standards of environmental protection.

This phase of development is now complete, details of capital and operating costs are known, and the technology is ready for the building of a commercial-size plant. There is little doubt that subsequent technological developments and the use of more sophisticated energy loops will enable still further reduction in cost.

In ten years time, very considerable advances in technology will have been made, and while the principles may not have changed, the productivity of that technology will have made a significant further advance. As a result of this technological learning curve, the cost of production of single cell protein will be very markedly reduced, and single cell protein will stand on all four legs in a truly commercial world.

ERRATA

PAG Bulletin Vol. VI, No. 2, June 1976

1. The legend for the cover picture should read as follows:

"At a center in the state of Bihar, India, children eat a nourishing meal prepared by mothers who attended demonstration classes given by nutrition workers. (UNICEF photo by Wolff).

2. Page 16, first column, paragraph 2, line 16 should read: "feeding of healthy infants in Chile (26)..."

PROTEIN-CALORIE ADVISORY GROUP

The Protein-Calorie Advisory Group of the United Nations System (PAG) is an interdisciplinary committee of internationally-recognized experts who advise the United Nations and its agencies on technical, economic, educational, social and other related aspects of global malnutrition problems and the broad programs and new areas of activity needed for combating them. Since its inception in 1955, the PAG has emphasized protein-calorie malnutrition as a primary and continuing threat to the health and survival of infants and young children in the developing countries and has played an active role in promoting the development of novel and locally-available protein resources for the developing world. The PAG also reacts to socioeconomic considerations, trends in world food supply and consumption and the need for governmental initiatives and priorities in dealing with these problems.

The PAG is sponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Bank for Reconstruction and Development (IBRD), and the United Nations.

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The purpose of the PAG Bulletin is to promote the exchange of information on the world malnutrition problem among all those who are motivated to work towards its solution. Published quarterly in English, French, and Spanish editions, it is sent gratis to individuals, institutions, and commercial organizations with an active interest in scientific, technological, economic or social aspects of protein-calorie malnutrition on a worldwide basis.

The PAG Bulletin can succeed in its mission only insofar as it can comprehensively and objectively communicate with its readership. Readers are invited to comment in writing on what they read in the Bulletin. In addition, the PAG Secretariat welcomes suggestions for broadening and deepening the scope of the Bulletin, thereby increasing its usefulness.

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VOLUME VI, NUMBER 4, DECEMBER 1978

PAG BULLETIN

Protein-Calorie Advisory Group
of the United Nations System

PAG Bulletin

Volume VI, Number 4, December 1976

COVER:

A map of the world indicating countries and territories where vitamin A deficiency is a public health problem. Based upon a recent WHO study. (The map was created specifically for use in the PAG Bulletin by the Cartographic Unit, Publishing Division, United Nations.)

IN THIS ISSUE....

PAG Bulletin, Vol. VI, No. 4, December 1976. *PAG Bulletin* is published quarterly in English, French and Spanish editions by the Protein-Calorie Advisory Group of the United Nations System, Room 606, 866 United Nations Plaza, New York, N.Y. 10017, U.S.A. Controlled circulation postage paid at New York, N.Y., U.S.A.

PAG BULLETIN

VOLUME VI, NUMBER 4
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VITAMIN A ENRICHMENT OF DONATED FOODS (WITH SPECIAL REFERENCE TO DRY SKIM MILK POWDER)*

Vitamin A deficiency and the xerophthalmia and blindness it leads to are serious nutritional problems with a considerable socio-economic significance in many developing countries. The other two are protein-energy-malnutrition (PEM) and nutritional anaemias. The frequent association of xerophthalmia with PEM increases the severity of both conditions and leads to severe morbidity and high mortality. The close link vitamin A deficiency has with defective immunological response and the consequent aggravation of childhood infections is another factor which contributes to its serious social significance.

The geographical distribution of vitamin A deficiency (see Annex I) is frequently found to overlap with those of PEM and nutritional anaemias. The three nutritional disorders particularly affect the vulnerable groups of those segments of the population most affected by the world's present economic hardships. Consequently, vitamin A deficiency is a problem in countries most eligible for food aid, given as a response to emergencies and/or as an investment needed for economic and social development.

Since vitamin A deficiency mostly affects young children, the UNICEF, with the advice of WHO, took upon itself the responsibility to enrich the dry skim milk (DSM) used in UNICEF's programs with vitamin A (and also vitamin D). This started some fifteen years ago and the use of enriched DSM by UNICEF has continued ever since. The Department of Agriculture of the United States of America authorized in 1965 the vitaminization of its DSM shipped as food aid under Title II (Food for Peace) of Public Law 480. The United Nations Protein-Calorie Advisory Group (PAG) issued in June 1972 the following explicit recommendation: "Evidence from WHO investigations and other sources clearly shows vitamin A deficiency to be a public health problem in developing countries in most parts of the world. It is especially severe in a number of countries in Southeast Asia where this deficiency is one of the main causes of blindness in children. One of the earliest recommendations of the PAG was that skim milk powder distributed by UNICEF should be fortified with vitamin A. UNICEF arranged to have skim milk powder donated to it by the U.S. Government fortified with relatively stable "beadlet" (microencapsulated) form. Soon afterwards, the U.S. Government adopted a policy of fortifying all skim milk powder donated under Title II (Food for Peace) feeding programs. Milk donated by European countries, however, is still unfortified. This is generally undesirable and is a potential hazard even if the skim milk powder is intended for a country where vitamin A deficiency is not a serious problem. Shipments of food are sometimes diverted for emergency use in populations

*Based on document No. WFP/CFA:2/7-D Add. 1 prepared by WHO, Geneva for use at the Second Session of the Committee on Food Aid Policies and Programmes, Rome, November 1976.

already deficient in vitamin A. In a recent emergency in Asia severe eye lesions and blindness were a serious problem which was exacerbated by distribution of unfortified skim milk powder. Standardized cereal-based mixtures which are fortified with protein concentrates, minerals and vitamins, including vitamin A, are in wide use under both normal and emergency circumstances. There is a risk, however, in any assumption that skim milk powder is always fortified with vitamin A. All skim milk powder used in feeding programs in developing countries should be so fortified; until this is done, the label should state that the product does not contain vitamin A.

The original PAG recommendation included vitamin D fortification and this has been done in the U.S. Government program. Since rickets continues to be a problem in a number of developing countries, it is recommended that skim milk powder be fortified also with vitamin D.

The levels recommended for stabilized forms of the vitamins are 1,500 mcg retinol (equivalent to 5,000 international units of vitamin A) and 12.5 mcg cholecalciferol (equivalent to 500 international units of vitamin D) per 100 grams of skim milk powder, assuming intakes in the range of 40 to 80 grams of skim milk powder per day."

The rapid progress made in the fields of physiology and of biochemistry of vitamin A has provided a coherent understanding of the metabolism of vitamin A and of its key role in visual perception. The evidence for the role and mechanism of vitamin A deficiency in aggravating human infectious diseases and infections in animals, (both in animal experiments and in veterinary medicine) remain to be established. It is not necessary, however, to await this evidence to make practical recommendations of public health importance.

Vitamin A enriched DSM in the context of food-aid and xerophthalmia

WHO strongly recommends that the above-quoted PAG stipulation be adhered to whenever feasible by every country donating DSM for use in food-aid programs. The enrichment of DSM with appropriate amounts of vitamin A is an essential safety step before the commodity is released as part of a food-aid grant. This is especially pertinent to the expanding operations of the World Food Programme at present, with its aid distributed more selectively than ever before to the least-developed countries and to those most severely affected by the present economic crisis. All these countries have the problem of PEM and vitamin A deficiency widely prevalent among the children.

In spite of the enlightened support for vitamin A enrichment received from the U.S.A. and the Netherlands, the amounts of vitamin enriched DSM available to the World Food Programme have not shown any increase in proportion to the needs. Therefore, the distribution of non-enriched DSM to children and women of child-bearing age unfortunately occurs leading to a great risk of development of xerophthalmia with its tragic outcome, impaired vision and blindness.

In dry hot climates, where green leafy vegetables are almost impossible to grow and where fresh fruit is not only a rarity but also beyond the economic reach of the majority, the vitamin A status of the vulnerable groups is often so poor that any incidental food shortage and/or infection will precipitate the eye lesions. A similar situation also exists in areas where vegetable provitamin A (β carotenes) or preformed vitamin A (mainly from whole milk and liver) are available, but not eaten due to ignorance of the value of these foods or as a result of some cultural traditions or taboos.

The mechanism triggering overt xerophthalmia

The meagre reserve of vitamin A in the

livers of children subsisting on deficient diets for prolonged periods gets rapidly utilized when some acute infection, particularly common infections such as measles, chicken pox, etc., overtakes the child. There is also at the same time a deterioration in the general nutritional status leading to a decrease in protein and energy reserves and the reserves of other nutrients. If this is allowed to proceed until the liver reserves of vitamin A become critically low, the vitamin content in the blood, which till then was carefully regulated, suddenly drops. Night-blindness and xerophthalmia promptly follow.

A satisfactory regulation of blood-level of vitamin A is a normal and delicate process which even helps those in a marginal or poor vitamin A status from developing symptoms of xerophthalmia until their liver reserves of vitamin A are practically exhausted. This explains why eye symptoms and signs are a late manifestation and the patient with eye lesions not immediately treated with vitamin A will almost certainly suffer impaired vision or even blindness. Moreover, the initial eye signs are usually very subtle. Anyone who has witnessed the examination of a crying sick child will realize that the eye signs can be missed, even by a careful and experienced physician. Expert ophthalmologists have shown beyond doubt that one or more among the early signs of xerophthalmia may not always occur in a set sequence either to give a warning signal or grant a respite but the damage occurs at explosive speed putting the eyes beyond the prospect of recovery.

The role of the vitamin A binding protein (Retinol Binding Protein, RBP) and the prealbumin, which constitute the transport system for vitamin A in circulating blood, is also significant in xerophthalamia. Although adequate amounts of vitamin A may be present in the livers of children suffering from protein deficiency, the level of vitamin A in blood could be low due to the lack of the transport system, which depends on efficient protein synthesis. With protein supplementation, the liver resumes synthesis of the transport protein, helping better liver mobilization of vitamin A leading to a rise in the blood level (Smith and Goodman[1], 1976). If the subject with protein deficiency happens to be in a marginal or poor vitamin A status with nearly exhausted liver stores of vitamin A, the provision of protein supplement will facilitate the liver synthesis of transport protein leading to mobilization and exhaustion of the already poor vitamin A reserve. In these individuals, therefore, an increase in the blood level of vitamin A means a rapid liver exhaustion of the nutrient, a real danger which should be avoided. The administration of a protein diet without a vitamin A supplement could, therefore, be dangerous in populations recognized to have very low liver reserves of vitamin A. It is unacceptable for anyone to await a field demonstration of this tragic effect in humans before undertaking vitamin A enrichment of protein-rich foods. Medical and health personnel have sometimes been implicitly guilty of causing preventable blindness in their well-meant but misdirected efforts to stimulate growth by feeding DSM, as a protein source, without provision of vitamin A supplement.

Feeding vitamin A enriched DSM to women of child-bearing age is also of benefit. During pregnancy, the growth of foetus and maternal tissues requires vitamin A. In vitamin A deficient circumstances it has been shown in Guatemala (G. Arroyave[2], 1976) that the breast milk from nursing mothers contains gradually less vitamin A (presumably their liver reserves are being depleted). If vitamin A fortified DSM is available to them, the vitamin A content of their milk could be either maintained or slightly increased. The significance of this observation is considerable because this allows the breast-fed infant to build

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1. Frank R. Smith and DeWitt S. Goodman (1976). Vitamin A transport in human vitamin A toxicity. New Eng. Jour. of Med. 294, 804.

2. G. Arroyave (1976). Rev. Centroamericana de Nutrición y Ciencias de Alimentos, Vol. I.

up his own reserve of vitamin A.

In Guatemala and in Costa Rica legislation has been passed recently[1] for compulsory fortification of sugar with vitamin A, to help increase vitamin A intake of the population. Both countries enrich their sugar with 15mcg of vitamin A per gram of crystallized sugar. Semi-yearly evaluations, thus far, have shown that this enrichment practice has benefitted the population, through increased vitamin levels in plasma and breast milk. The availability of this source of vitamin A is bound to help the nursing mothers not only improve their own nutritional status but also those of their nursing infants. The experience of Guatemala and Costa Rica is too short to allow its evaluation in terms of xerophthalmia, impaired vision and blindness.

Enrichment policy, present and future

Among populations receiving food aid, many live in countries or areas where xerophthalmia is widely prevalent in the vulnerable groups among the less privileged. Should food-aid be allowed to add--however little--to the existing risk or should it help prevent a serious problem by offering vitamin A enriched donated foods used as protein sources and particularly the donated DSM. Blended protein-rich foods, such as CSM (corn-soy-milk) and WSB (wheat-soy-blend), are vitaminized, and the issue seems to be practically confined to DSM.

Xerophthalmia-induced blindness is not a trifling issue. In India alone 10,000 to 12,000 people (mostly children) go blind every year due to xerophthalmia. The generally agreed estimate is that 100,000 people are blinded in the world by xerophthalmia every year. This estimate should be seen in perspective: for every xerophthalmia case totally blind, two more become blind in only one eye, or suffer from corneal scars which seriously impair sight. In several African countries, xerophthalmia is regarded as a curse and out of shame the parents often conceal blind children who are consequently missed in a survey. Parental and sibling neglect seldom allows the xerophthalmic child to survive his blindness for more than a couple of years in Kenya (Sauter[2], 1976).

Since adequately enriched DSM can effectively prevent xerophthalmia, it is the moral obligation to prevent it wherever a food-aided program provides the opportunity to do so. As mentioned above, DSM is not the only commodity which can be enriched to supply the needed vitamin A supplement: several others - such as cereal products, sugar, tea and even the condiment monodosium glutamate - are also being tried as a vehicle to introduce the needed vitamin in deficient diets. The choice is ultimately governed by locally prevailing feeding habits and by the existence of some convenient focal point in the food preparation process where the controlled vitamin enrichment can cheaply and conveniently take place. Such a focal point exists at the spray-drying stage in the manufacture of DSM and if it is generally used, the cost of vitaminization is approximately 2/3 per cent of the present price of DSM (over $2 per $350 to the metric ton). The extra expense involved in adding simultaneously vitamin A, as recommended by PAG, will be small. UNICEF's experience from the time when it did its own enrichment by the dry process over ten years ago shows the cost to be an average of just over 1 per cent. The dry process adopted was expensive even at the time when the price of DSM was lower and that of vitamin A higher. It was also cumbersome to pack non-enriched DSM at source, unpacking it for dry process enrichment later on and repacking it. It will be very much cheaper for a manufacturing country to enrich all of its food-aid donations than to do it only for those consignments destined to countries or areas where

1. Honduras has also legislated to enrich sugar very recently in October 1976.

2. J.J.M. Sauter (1976). Xerophthalmia and Measles in Kenya. Published by Drukkery Van Denderen, Groningen, Netherlands, 1976.

threatening xerophthalmia demands enrichment. This, together with the fact that no over-dosage of vitamin A was reported as a result of vitaminized DSM consumption (the same being true for vitamin D in developing countries), is a strong argument in favor of WHO's recommendation to have a generalized enrichment rather than specific enrichment only for groups at risk. Since non-enriched stocks exist today, the transition from the present situation to the preferred generalized enrichment will necessarily take a couple of years. During this transition period, WHO recommends that all DSM should invariably be vitaminized at source if meant for direct distribution for countries where xerophthalmia is a recognized public health risk. This practice should be followed eventually for every country of destination. All non-enriched DSM should be addressed specifically to countries where xerophthalmia is not a public health risk, or to dairy plants or dairy grids with the explicit agreement that the plants or grids will themselves add vitamin A in accordance with their consumers' needs.

The hazards of such an approach have not escaped notice, especially as shipments are often diverted for good reason from their original destination to some other where the unenriched DSM could spell disaster. To minimize the danger, WHO recommends that each package of DSM be clearly labelled as "Non-enriched" or "Enriched" as the case may be. Some experts have even suggested that all non-enriched DSM should be labelled as unfit for human consumption. While the intended safety factor in this suggestion is appreciated, adoption of the same may have other disadvantages such as embarrassment to dairy plants or grids and/or creation of confusion when batches of DSM may have become unfit for human consumption due to special field circumstances.

In some cases of emergency relief operations, when non-enriched DSM was directed to xerophthalmia-prone countries, WHO recommended, and sometimes even supplied, vitamin A capsules as a xerophthalmia-preventive measure. This is not WHO's preferred procedure as it is expensive, puts an extra management burden on the recipient community and is seldom a reliable way of achieving equitable vitamin A distribution. A properly chosen enriched food is better in such respects, and vitamin A preparations in the form of solutions or capsules should be saved for the early treatment of recognized cases of xerophthalmia.

WHO is convinced that the need for vitamin A enriched foods is one that will be with us for the next several decades. Nevertheless, WHO does not consider that such enrichment is the ultimate method of preventing deficiency diseases. With gradual progress in development and implementation of food and nutrition policies together with persuasive health and nutrition education and improvement of diet, the deficiency should finally disappear. In this respect, WFP-supported projects leading to domestic or regional production of nutritious foods are certainly one of the long-term health-promoting activities of the Programme. The red palm oil project in Ghana is a step in this direction as it could ultimately provide the provitamin A - β carotenes, badly lacking now throughout the Sahelian area.

Xerophthalmia complicating measles as a cause of blindness

It is widely believed that blindness due to xerophthalmia is the consequence of measles, rather than the result of vitamin A deficiency per se. Irritation of the eyes and nose is a common symptom in measles and is frequently seen at onset. Examination of the eye membranes with the help of selective vital stains and proper magnification has shown that measles is regularly accompanied by the specific eye lesions of kerato-conjunctivitis. These are normally so benign that they disappear spontaneously and completely usually in about five days, although in some exceptional cases, superficial eye lesions could be observed for as long as two months. These measles lesions do not leave scars which impair vision. As the patient tends to avoid light when the eye irritation is present in measles, one may naturally suspect measles having initiated a process in the eye leading

to blindness. However, in well nourished patients measles is invariably a mild disease of no permanent consequence to eyes or sight, irrespective of the patient's age, sex or ethnic origin. In malnourished groups, however, the disease is so serious that nutritionists often gauge the nutritional status of a community from mortality from measles. Malnutrition and measles act synergistically, aggravating the symptoms and signs of each other leading to high mortality. If in such a situation the patient's liver is poorly provided with what should be a safe reserve of vitamin A[1], the acute malnutrition would precipitate xerophthalmia as can be seen from the following table:

Relation of Liver Stores to Eye Signs of Vitamin A Deficiency in Malnourished Children[2]

Diagnosis	Xeroph-thalmia	No. of cases	Mean Liver Vitamin A (mcg/g)
Kwashiorkor	absent	22	17.5
Kwashiorkor	present	17	6.7
Marasmus	absent	32	14.6
Marasmus	present	10	6.6

Forty to 100 mcg of vitamin A per gram of liver tissue is commonly reported in healthy children and the low and very low values in the table show that in malnutrition liver reserves are nearly exhausted. It is evident that the use of vitaminized DSM in the treatment of malnutrition may make all the difference between sight and blindness.

The recent study by Sauter in Kenya has again shown that in well-nourished children measles is of no consequence as a cause of blindness. When a measles epidemic occurred in 1974 in Kenya, xerophthalmia was found in all areas where white maize was used as the staple diet. Unless one has unravelled the triggering effect of measles and the mutually aggravating roles of measles, malnutrition and vitamin A deficiency, one would be tempted to consider measles as the apparent obvious cause of blindness. It is noteworthy that several African populations are aware of the heavy mortality of measles as a common popular saying advises: "Don't count your children until they have had measles."

Conclusions and recommendations

It is extremely desirable to enrich dry skim milk with the recommended amounts of vitamin A, particularly in the present context of food aid. Such enlightened policy will certainly save the sight of many children who might otherwise have been among the 100,000 xerophthalmia-caused blindness every year. Food aid programs now devote nearly 75% of their resources to countries where the problem of vitamin A deficiency is widely prevalent. If the food commodities used in this program are vitamin A enriched, it would save the eyes of several thousand every year.

Although it is appreciated that the transition from the present situation to generalized enrichment of DSM will initially require effort and expense, once established, it would cost less than the handling and distribution losses which occur even in the most efficiently managed food-aided operations.

1. "Safe reserve" of vitamin A is a recent concept. It is now possible to predict from the liver reserve level for how many days a vitamin deficient diet may be harmlessly tolerated.

2. From McLaren, D.S.: In: "Vitamin A Deficiency and Xerophthalmia" (1976). (WHO Tech. Rep. Series No. 590).

Annex I.

Tentative List of Countries and Territories where Vitamin A Deficiency is a Public Health Problem*

Afghanistan
Antigua
Benin
Bangladesh
Bolivia
Brazil
Burma
Cape Verde
Chile
Colombia
Comores
Democratic Kampuchea
Democratic People's Republic of Korea
Dominica
Ecuador
Egypt
El Salvador
Ethiopia
Fiji
Ghana
Grenada
Haiti
Honduras
India
Indonesia
Iran
Iraq
Jordan
Kenya
Lao People's Democratic Republic
Lebanon
Libyan Arab Republic
Malawi
Malaysia
Maldives
Mali
Mauritania
Mozambique
Nepal
Nicaragua
Niger
Nigeria
Pakistan
Panama
Papua New Guinea
Peru
Philippines
Republic of Korea
Rwanda
Sao Tome and Principe
Senegal
Socialist Republic of Viet Nam
Somalia
South Africa
Southern Rhodesia
Sri Lanka
St. Kitts - Nevis & Anguilla
St. Lucia
St. Vincent
Sudan
Syrian Arab Republic
Thailand
Togo
Tonga
Tunisia
Turkey
Tuvalu
United Republic of Tanzania
Upper Volta
Uganda
Uruguay
Zaire
Zambia

*The list is meant to guide vitaminized DSM to those areas where it is most needed. Nationally-held stocks are often distributed and redistributed unpredictably. Hence the list includes every country where the existence of xerophthalmic area(s) has been reported, even if it be a small area of the country. Guatemala and Costa Rica are not listed because of the on-going vitamin A enrichment program. Honduras has also passed a similar legislation in October 1976.

CHANGE IN PAG SECRETARIAT

Dr. P.S. Venkatachalam, a WHO staff member, who served as Assistant Scientific Secretary of PAG from 1970 to 1976, will be leaving the Secretariat in February 1977 to join the UN University in Tokyo.

THE PAG SYMPOSIUM ON HYDROCARBON-GROWN SCP PRODUCTS FOR ANIMAL FEEDING

Several papers presented at the Symposium were published in the PAG Bulletin, Volume VI, No. 3, 1976. The following pages of this issue carry some more papers given at the Symposium. The PAG will issue separately the proceedings of the Symposium including the discussions, at a later date.

THE ROLE OF SCPs GROWN ON HYDROCARBONS FOR ANIMAL FEEDING

J. C. Senez*

The first question that arises when the economic prospects for SCPs are evaluated is, of course, the size of the potential market. In this connection, Lord Ritchie-Calder--in a particularly authoritative and brilliant fashion--has shown earlier what can be expected of SCPs as animal feed. However, this paper will begin with a few brief reflections on world protein requirements and probable medium-term trends in those requirements. It should be stressed that this problem arises in very different forms, depending on whether animal nutrition or direct human nutrition is being considered. Nevertheless, these two aspects are closely related.

FAO statistics show that present world consumption of protein for human nutrition is 91 million tons, which at first sight appears to match world requirements almost exactly when calculated on the basis of a total population of 4.5 billion at a daily per capita protein intake of 70 grams. However, closer examination reveals that the availability of such proteins is very unevenly distributed. The 1 billion inhabitants of the industrialized countries alone consume 37 million tons, yielding an average daily intake of 96.4 grams. On the other hand, the 3 billion inhabitants of the developing countries get only 53 million tons - an average protein intake of only 57 grams. As matters stand, therefore, production for the third world would have to expand by 12 million tons in order to achieve a normal protein intake and by 36 million tons if a level of consumption comparable to that of the wealthy countries is to be attained.

The disparity between rich and poor countries is even more marked if one considers not the quantity of all proteins available but the quantity of high-quality proteins, i.e., animal proteins or those having a similar composition in terms of essential amino acids. Of the 110.3 million tons of meat and fish currently consumed, 62.2 million tons (56.4 per cent) are consumed by the industrialized countries and only 48.1 million tons (43.6 per cent) by the rest of the world.

As Table I shows, projected protein demand from 1970 to 1990 taking into account population growth and increased individual consumption can be expected to rise by 26 per cent in the industrialized countries and, it is to be hoped, by 82 per cent in the third world. On the basis of these data the goal for the period in question is an increase in world protein production at 59%, i.e., by about 54 million tons.

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Animal Feed Demands

To the present and projected demand for direct human nutrition must be added the demand for animal feed purposes. The bulk of the protein used for animal feed is supplied by cereals, which contain about 10% protein. As Table II shows, 42% of the present cereal supply is used for livestock and in the industrialized countries cereals consumed for this purpose alone amount to nearly 80% of that used by the rest of the world for direct human nutrition.

Between now and 1990, the rich countries' demand for animal feed purposes alone is expected to increase by a further 200 million tons and a considerable share of this additional supply will have to be obtained on the world market. At the same time, as a result of population growth, the third world's vital cereal requirements for human nutrition will have increased during the same period by 300 million tons. Furthermore, FAO forecasts that in the third world itself demand for cereals for animal feed will grow by 100 million tons. This forecast-which assumes a rise in living standards as well as a substantial increase in production-may well be proved wrong by actual developments. In any event, however, cereals can be expected to be the object of fierce competition between rich and poor countries, in upcoming decades, the latter trying to survive and the former to improve or at least maintain their present nutritional level.

Protein Supplements for Livestock

In addition to cereals, livestock feeding in the industrialized countries consumes enormous quantities of protein supplements, mainly in the form of fishmeal, powdered milk, and most of all, oilseed cake, chiefly that based on soybeans. The figures for protein consumption for animal feed in the nine Common Market countries illustrate this point. Of the 15 million tons consumed in 1972, 6.8 million tons (45%) came from cereals and 8.2 million tons (55%) from various sources of protein supplements. However, although the European Community produces 83% of the cereals it uses for livestock feed, it has to import 85% of its supplemental proteins, including all of the some 7.5 million tons of soybeans it consumes, at a cost of about $1.2 billion annually.

Europe cannot hope to produce enough soybeans for its own needs. The dry summers typical of the European climate are unsuitable for this crop. Furthermore, Europe is short of arable land. It is estimated that France, in order to supply its own protein requirements, would have to reduce by one third the area currently under cereal crops. That country's choices in this matter are governed by the fact that in France, as in the rest of Western Europe, the quantity of soya and other legumes produced could at most reach 70% the level attained for cereals.

Regional Economic Impediments

A final major obstacle to the production of vegetable proteins in Europe is the fact that fragmentation of land holdings and high costs for arable land compel farmers to concentrate increasingly on fruit production and market-gardening rather than on extensive agriculture.

In addition, the dependence of the European countries on external protein supplies imposes a heavy burden on their trade balances. In the case of France, for example, the yearly expenditure on soybean imports is equivalent to the total export earnings of the automobile industry. One wonders how much longer France and other European countries can tolerate this drain on their foreign reserves, especially when considering the pressures imposed by imports of petroleum and other raw materials.

Such costs, which are already staggering, are increasing sharply. European protein consumption for animal feed is rising by a steady 8.3% a year, thus doubling every 12 years. The economic crisis has temporarily halted this rise, but the curve seems poised to

resume its upward course.

A rise in living standards is inevitably accompanied by an increase in consumption of animal proteins and a decline in cereal consumption. The current distribution of animal and vegetable protein consumption in various parts of the world shows that Western Europe still has a far lower level of meat consumption than North America, Australia or New Zealand. In those countries, however, the trend in question has not yet reached its peak. In the U.S., consumption of meat and poultry increased by 21% between 1960 and 1972, almost as fast as the growth in energy consumption which rose by 34% during the same period.

It is clear, therefore, that Europe does now and will in the future increasingly depend for its existence on a world foodstuffs market, the capacity of which to sustain for much longer, such a rapidly rising demand, is in doubt.

The Socioeconomic Factor in World Hunger

The present international trade volume in cereals is about 10 million tons, almost all of which is absorbed by only two countries, the U.S.S.R. and India. This does not mean, of course, that production and requirements are in balance everywhere else, but rather that international trade is limited by the supplies available from the exporting countries, shortage of transport and, above all, by the sheer poverty of the undernourished populations. In this connection, it cannot be too strongly emphasized that world hunger is primarily a socioeconomic problem.

Further, it must be noted that world trade in foodstuffs is dominated by two countries, the U.S. and Canada, which together supply 78% of all available cereals. The preeminence of the U.S. on the international market is even more marked in respect of proteins. As Table III shows, the U.S. produces 44 million tons of soybeans, of which 43% is exported, mainly to Western Europe and Japan. The only other major producer, China, consumes the whole of its output. Thus, until last year, the United States exerted a virtual monopoly in the world market. This situation is changing somewhat as a result of the rapid growth of soybean cultivation in Brazil, which produced approximately 10 million tons in 1975 and is no doubt destined to become a major exporter in the near future. Nevertheless, it remains that U.S. exports of foodstuffs amount to some $10 billion annually equivalent to about one fourth of the French national budget. These exports alone offset the cost of all imports of raw materials, including petroleum, and are an essential factor in the United States' economic strength.

North America's overwhelming domination of international trade in foodstuffs gives rise to some problems. First, the fact that almost all commodities marketed come from a single geographical region brings with it the risk that if unfavorable climatic conditions and poor harvests should cause the U.S. and Canada to suspend their exports, Europe and other regions dependent on these supplies would suddenly find themselves in critical straits. Secondly, control of the foodstuffs market is obviously a powerful instrument of political pressure, a fact of which the U.S. itself, where references to the concept of the "food weapon" are becoming commonplace, is fully aware. In that respect, the U.S. temporary embargo on soybean exports in 1973 should give European governments food for thought.

Prospects for the Expansion of Protein Resources

From this brief analysis of the prevailing situation and future prospect, it will be seen that it is imperative for both the industrialized countries and the rest of the world to expand their own food production, and particularly their protein resources, without delay.

In approaching this problem, the first question

obviously must be what the potential of agriculture is. It might be thought that progress in mechanized farming, fertilizer production and the improvement of plant varieties warrant high expectations. However, recent statistics of FAO and the U.S. Department of Agriculture show that although world food production has indeed increased in the last 15 years, it has barely kept pace with population growth. Although per capita food production is continuing to rise in the industrialized countries, it has remained static in the third world, and has even declined overall by about 6 per cent on the African continent. Consequently, on the basis of individual consumption, the world food situation has not improved but has at best stayed the same, and the disparity between rich and poor nations has become more marked.

The medium- and long-term prospects are even gloomier. World agricultural development is hampered by socioeconomic and political constraints of increasing difficulty. Furthermore, there are natural limits to this development which, in the view of several highly-qualified experts, will shortly be reached. One of the main arguments advanced in support of this theory is that almost all arable land on the planet is already under cultivation and that the clearing of the Amazon forest or other unutilized regions cannot be relied on to increase the area of arable land significantly. Equally, disquieting--if not more so--is the scarcity of fresh water, which limits prospects for irrigation. Finally, there are limits to agricultural productivity, and, according to such experts as M.D. Miller of the University of California, the biological limit of maize productivity has already been reached in the U.S.

Nevertheless, the most serious threat to the medium-term development of agricultural production is an economic one. As a result of the energy crisis and world inflation, the food situation will inevitably deteriorate, and do so rapidly. Before the rise in oil prices, energy was already held to account for between 20% and 30% of the cost of agricultural commodities. This percentage has certainly risen considerably since that time, a fact which will undoubtedly have repercussions on the prices of agricultural commodities in the near future.

Either agricultural prices, which still lag far behind industrial prices, will soar as well, or farmers--and in particular those with small and medium-scale holding on which, in the final analysis, most of the output in both Europe and the developing countries depends--will no longer be able to invest sufficient capital to maintain production at its present level. The most likely prediction is that there will be a combination of these two alternatives and that, in the near future, the world will witness both a decline in production and a rise in prices.

SCP Production from Petroleum Hydrocarbons

It is against this background that the enormous potentialities of single cell protein (SCPs) for animal feeds would be considered. The most outstanding among the SCPs are those produced from the paraffin series of hydrocarbons. At the moment, yeasts grown on alkanes are the only SCPs produced on an industrial scale whose high nutritional value and safety for animal feeds have been fully demonstrated and which have been marketed successfully. It is to them that the emergence of SCP from the realm of science fiction is to be attributed. In fact, they are still the only SCPs which can currently be produced on a scale commensurate with global needs.

If crude petroleum is reckoned to contain an average of 7% of paraffins which can be converted into an equal weight of dry yeast, the 2.5 billion tons of petroleum now extracted and consumed throughout the world could yield 175 million tons of yeast, i.e., more than 110 million tons of protein annually. In other words, the application for this purpose of only 7 per cent of current petroleum consumption could alone more than meet all projected new protein requirements until the year 2000; these needs, as has been shown, certainly could not be met by

agriculture alone.

Some may question the future of petroleum as a source of protein at a time when the cost of this raw material is increasing by leaps and bounds and when its exhaustion over the medium term is considered inevitable. Such fears are unjustified. As far as the availability of petroleum is concerned, it has been pointed out earlier that a relatively tiny proportion of the petroleum quantities consumed throughout the world would be sufficient to meet all protein needs. Furthermore, the alkanes used for protein production are not among the most valuable constituents of petroleum and sometimes have to be removed in any event in order to produce certain crude oils--such as those from Libya, which are particularly rich in paraffins--making them suitable both for energy use and petrochemical production. Furthermore, necessity is supreme, and it may not be too soon to wonder whether the precious petroleum reserves remaining should not be used for the production of food proteins and for petrochemicals, rather than be burned in internal combustion engines and furnaces.

A more disturbing matter, at least at first sight, is the rising price of petroleum. However, two observations are called for in this connection. Firstly, the cost of the raw material is by no means a major component of the cost of yeast grown on alkanes--it is actually lower than the costs of technological constraints such as refrigeration and aeration of the cultures, the harvesting and processing of the cells and the amortization of plant. Another important consideration is that, as already pointed out, the price of petroleum must inevitably have repercussions of the same, or a greater order of magnitude on the cost of conventional proteins.

Prospects for the SCP Industry

One might reasonably ask, then, why the SCP industry has not enjoyed greater success, particularly in Europe, since it clearly represents a means by which that continent could largely free itself from its vexatious dependence on imported animal feed proteins.

One reason is that problems exist regarding licensing and acceptability. The second reason is economic. As in the case of any other new industrial product, it must be demonstrated that SCPs are not only useful and of high quality, but also competitive and economically viable. In this connection, the wide fluctuations currently occurring in the reference costs of conventional proteins and industrial investment make any short-term forecast impossible. It is impossible to determine the manufacturing cost of SCPs with any accuracy just now.

In fact, the main cause of uncertainty with regard to the profitability of industrial proteins is the price of reference proteins, especially soybeans. In the connection, it is surprising that, in spite of worldwide inflation, the price of soybean proteins fell from between 1.23 and 1.35 francs per kg in October 1974 to between 90 centimes and 1 franc in July 1975, and has held at the same level or below ever since.

This completely abnormal, and undoubtedly temporary, situation is partly due to an abundant harvest, the depreciation of the dollar and to some falling off in market demand as a result of the economic depression. Another factor is that the U.S. appears to be maintaining the price of soybean cake at its present artificial level by means of compensatory measures in favor of oil--the other product of this highly profitable plant. Although the price of soybean cake is the same today as in 1972--which, taking account of inflation, corresponds to a real drop of 70%--the price of soya seed has usually followed economic trends. From $2-$2.50 a bushel in 1972, the price has risen to between $5 and $6 in 1976, thus having more than doubled. It is clear that oil makes up the difference and there are grounds for wondering to what extent this represents a U.S. policy of trade deterrence whose objects are Brazilian soybean and SCPs. In any ever

it is doubtful whether the price of the oil can offset the cheap cake prices for much longer or make the growing of soybean sufficiently profitable for U.S. farmers to abandon their preference for maize or wheat production.

At all events, the present situation cannot continue indefinitely, and, sooner or later, as we have seen, rising energy and investment costs together with an inevitable increase in demand, must have serious repercussions on all agricultural prices, including soybeans.

It is nevertheless true that, for the moment, the SCP industry is obliged to mark time until its profitability can be firmly established. In the interval, however, valuable time may be lost. Under the pressure of population growth and the -impending threat of a world food crisis, industrial production of protein-particularly for use as additive in animal feeds-will soon become an absolute necessity. In the light of these facts, the object lesson provided by the energy crisis should not be forgotten. As everyone knows, the development of atomic energy was held up for a long time because of doubts about its profitability; when the need for it finally became obvious, there was anguished uncertainty as to whether it was already too late.

Conclusion

It is in the nature of things that the approaching food crisis will not materialize all at once, but rather will accrue in the form of alternating scarcity and apparent abundance, a cycle that is being experienced at present. These ups and downs, which are due to the complex influence of financial speculation and to changes in the relative strength of producer and consumer nations, induce a false sense of security and may dangerously delay the implementation of measures that are both urgent and essential. It is to be hoped that governments in Europe and the rest of the world will not fall into the trap and will realize in time the need to make the industrial development of SCPs one of their immediate priority objectives.

Table I.

Projected Trend in Population and Demand for Edible Proteins for Direct Human Consumption

(Based on preparatory documents for the World Food Conference, 1974)

	1970		1985		1990		Increase 1970-90
Industrialized countries	1072*	(37.7)**	1227	(44.8)	1277	(47.5)	26%
Third World countries	2542	(53.4)	3631	(83.9)	4069	(97.4)	82%
Total	3621	(91.1)	4858	(128.7)	5346	(144.9)	59%

* Population in millions
**Demand for protein (millions m. tons)

Table II.

Cereals: World Food Demand

	(million m. tons)				
	1970	1980	1985	1990	Increase 1970/1990 (per cent)
Industrialized countries:					
Food	160.9	163.1	164.1	164.6	2.3
Feed	371.5	467.9	522.7	565.7	52.3
Total	532.4	631.0	686.8	730.3	37.1
Third World countries:					
Food	476.8	609.8	689.7	772.5	65.1
Feed	50.9	99.6	127.3	163.3	320.8
Total	518.7	709.4	817.0	935.8	80.4
World totals:					
Food	628.7	772.9	853.8	937.1	49.0
Feed	422.4	567.5	650.0	729.0	72.5
Total	1051.4	1340.4	1503.8	1666.1	58.4

Table III.

Soybean Production

	(million m. tons)	
	World	U.S.A.
1940	12.65	2.06
1950	17.94	7.65
1960	27.06	14.12
1970	45.88	20.00
1972	60.00	44.12

N.B.: U.S.A.: Area under cultivation 22.7 million hectares - proportion of production exported: 43%. China: 11 million tons (production stable); Brazil: 1971: 2.22 million tons; 1975: 10.0 million tons.

THE TOXICOLOGICAL ASPECTS OF SINGLE CELL PROTEINS USED IN ANIMAL FEEDING

R. Truhaut* and R. Ferrando**

It is becoming increasingly clear that any toxicological evaluation must subsume detailed knowledge of the diet on which the evaluation is based. The slightest error, the slightest imbalance, may cause the analyst unwittingly to draw erroneous conclusions. This observation applies even more strongly in the case of an assessment of the virtues and defects of new foods such as the protein-rich products derived from various microorganisms. In such products, the good and the bad may be closely associated with each other, and may mask or reinforce each others' effects. An excess may thus exacerbate an imbalance, an effect which may be mistaken for an intrinsic harmful property of the food under examination. To use a new food in a volume disproportionate to the nutritional balance of a ration designed for a given species leads, especially when the efficiency ratio of new proteins is being assessed, to a considerable fall in protein efficiency. The desire of the toxicologist to learn how far it is possible to go is understandable, but it is possible for the nutritionist to discover how, in such an attempt, excess has brought about serious imbalances which may alter metabolism. It is essential that such studies should be inspired by a spirit of "critical biology", to borrow the phrase used by Lefebvre as the title of one of his works. Complete documentation on the substrate, the microorganism used--particularly its absence of pathogenic properties--the technology employed in causing one to exploit the other, and finally a detailed chemical and microbiological analysis of the composition of the end product should make it possible to devise, with the full assent of both toxicologist and nutritionists, guidelines for the studies to be carried out with laboratory animals.

It should be borne in mind throughout this process that in practice there are three constraints on the use of such new sources of protein in the feed rations of livestock:

1. The safety requirement for livestock and thereafter for consumers of livestock derived products. In any event, novel sources of protein may well be used at a later stage in human feeding.

2. The requirement of exact knowledge of the biological value of these proteins.

3. The economic requirement--that is, the unit cost of the protein as compared with that of protein from traditional sources; the price can be reduced or increased depending on the supplementation value of the product concerned within a standard feed ration.

The likelihood is that the information on the substrate, the microorganism employed, the technology used in preparing the protein and the detailed analysis of the product when ready for use in feed, will serve to guide the organization of the tests. The economic requirement, which is dependent on the biological value, governs the percentage of the new food employed and accordingly affects the safety aspect.

Ascertaining Toxicity

As PAG experts have affirmed, it is just as illogical to argue that just because some microorganisms are toxic, all products derived from microorganisms are unsuitable for human and animal consumption. To push this argument to its logical conclusion would

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mean adopting the same attitude with regard to plants and mushrooms. All that needs to be done is to distinguish the good from the bad. In the case of our customary "traditional" food, this distinction was made empirically, slowly, imperfectly, by means of famines and recorded cases of poisoning. We can and should operate on a scientific basis and make the distinction quickly, surely and accurately for novel foodstuffs.

What information and which aspects should be relied on in conducting research for the purpose of eliminating all risks in the use of SCPs in animal feeding?

The real or merely apparent risks may arise from a great many contingencies, the main ones being:

1. Imbalance of proteins and presence of undesirable nitrogenous compounds.

2. The presence of residues and fractions of the substrates used in the cultured micro-organism; nature of the lipids of the micro-organism.

3. Technological processes which may affect the toxicity of the end product.

4. Presence of polycyclic aromatic compounds.

5. Presence of heavy metals.

6. Decomposition of microbiological origin.

The list of contingencies is not exhaustive, but it applies equally to traditional foods.

Imbalance of Proteins

Every food shows imbalances among its nitrogenous compounds when compared with egg protein. In the case of yeasts there is a sizable deficit in sulfur amino acids, and these have to be added in order to ensure proper protein efficiency. Without such adjuncts, alkane yeasts have a protein efficiency of between 0.41 and 0.66, according to tests by one of the authors. In the presence of a supplement of 3 per cent of DL-methionine, these protein efficiencies rise to, respectively, 1.86 and 2.39. Without any DL-methionine supplement, feeding stuff yeasts from brewery which have never before been subjected to expert analysis, have a virtually nil protein efficiency.

Dried blood, used as an ingredient of animal feed, also has a nil protein efficiency. Its use to supply 10% of the crude protein content (CPC) of the ration designed to measure protein efficiency caused mortality as high as 22% in certain experiments with rats. The cause is the well-known leucin-isoleucin imbalance of dried blood and, to a lesser degree, the deficiency of sulfur amino acids. Yet nothing is more natural than blood!

Amount Fed in Ration and Its Significance

Reverting to the requirements for research on novel single cell protein sources, it is essential that the dosage of the product added to the rations of the test animals should on no account be exceeded for the sake of demonstrating one or more toxic properties of those products. The figures in Table I illustrate the potential danger of this practice for interpreting the results.

It is essential, in our view, when a non-conventional food is being studied, to examine its effects when used in the proportions which are customary in farming practice. If there is a risk that a given residue is present in this new food, the experimenter should confine himself to specifying an analytical ceiling for such risk. In order to do so, the principle of the acceptable daily intake (ADI) will be applied, but not the results of tests which amount to distributing to the test animals unduly high levels of the food considered, which could distort the results. An excessive intake may, in the same way as a deficiency of protein in the ration, completely alter the reactions of the organism to a given toxin. A balance must always be achieved by avoiding both profusion and deficiency. Moreover, any measurement of

weight gain and of consumption index should be accompanied by a calculation of protein efficiency and a determination of the serum urea level. These two parameters provide a clear indication of protein imbalance in the ration. The measurement of uraemia (serum urea level) might even suffice in some cases since, in the experiment reported on in Table II, the correlation is as follows:

$y = 6.492\ x + 3.70$
where y = protein efficiency
x = urea (mg/l)
so that r = 0.864 (highly significant)

The authors have already drawn attention to this fact in the case of sheep (Ferrando and colleagues, 1970), and Taylor and colleagues have recently observed it among young men (1974).

The nucleic acids content varies--in some single cell or multi-cell organisms--from 4.25% to 18%, but these acids create no problem for livestock, and among domestic animals only Dalmatian dogs may be endangered if they consume them in small quantities.

It is quite a different matter, of course, for human beings. In that instance, it must be noted that during fermentation toxic amines or polyamines may be produced. This question will be discussed in connection with the possible effects of technological processes. Moreover, in the course of research on toxic effects, the presence of lysinoalanine, which caused renal lesions in rats, was detected in protein concentrates prepared from soybeans. On the other hand, Sternberg and colleagues (1975) have just discovered that this amino acid exists in eggwhite, milk and frankfurters in amounts ranging from 50 to 6,900 and even 50,000 µg per gram. Human beings have therefore been consuming lysinoalanine for a long time without ill effects. Recent work has shown that the rat is specifically sensitive to lysinoalanine but not other species.

The Presence of Substrate Residues and Fractions Including Lipids of the Micro-organism

These residues and fractions of substrates can be relatively abundant. From the public health viewpoint, the most important of them are the polycyclic aromatic hydrocarbons, the n-paraffins and the fatty acids with an odd number of carbon atoms. It is known that these substances occur in varying but often significant amounts in natural foods.

Instant coffee, according to Strobel (1974), contains 0.02 to 0.06 µg/kg of 3:4 benzopyrene. Lucisano and colleagues (1973) report that in sausage manufacture in Italy the 5 µg/kg tolerance is exceeded in salami; they add that in Italy benzopyrene is not considered a "health hazard".

For cereal grains - foods normally consumed by both livestock and human beings - the figures are:

Wheat and barley in industrial regions: 0.29 - 1.2 µg/kg (Soos; 1974);

Wheat and barley in non-industrial regions: 0.15 - 0.3 µg/kg (Soos; 1974);

Cereals from various regions: 0.2 - 7.5 µg/kg (Kolar and colleagues; 1975);

Vegetables from various regions: 0.1 - 24.3 µg/kg (Kolar and colleagues; 1975).

Graf and Diehl, cited by one of the authors (1972), find 6.60 µg/kg of 3:4 benzopyrene for leeks, 12 for lettuce and 50 for endives. The content of polycyclic aromatic compounds in certain yeasts is given in Table II. Taking only the data for alkane yeasts and using the total of the polycyclic aromatic compounds in the Grangemouth sample with the highest content, the total is 4.3 µg per kilo dry weight. The proportion of these yeasts used in pig or poultry feed is 10-15%. In one kilo of these feeds, therefore, there will be between 0.43 and 0.65 µg or polycyclic aromatic compounds. Grains, which make up 65 to 75% of these mixtures, will

add polycyclic aromatic compounds at rates of between 19.5 µg and almost 50 µg per kilo of feed, depending on the grain content and derived from various sources. Comment would be superfluous, except to point out again how strictly novel protein sources are treated. PAG Guideline No. 15 and technical report No. 12 of IUPAC[1] in fact propose the following dry weight tolerances for the residual hydrocarbons which may be present in proteins derived from microorganisms grown on alkanes:

Total residual hydrocarbons < 0.5 per cent
Total residual aromatic hydrocarbons
<0.05 per cent
Benzopyrene <5 µg/kg[2].

According to a study published by WHO, a daily oral intake of 0.04 µg/kg of benzo(a)pyrene per subject creates a risk of carcinogenesis of 2×10^{-5} [3].

The nature and toxicity of residual lipids or those produced by microorganisms grown on certain substrates for protein production are still the subject of controversy. A number of studies show, however, that the figures for n-paraffins accumulating in the liver and fat of animals consuming yeasts grown on gasoil do not differ from those found in the controls. They are, moreover, at the outer limit of analytical precision (5 ppm), as is shown in several licensing applications. Further, the n-paraffin content of yeasts grown directly on this substrate should not exceed 0.5%. Nevertheless, a certain quantity of residual paraffins (<1 per cent) is found in the fat of animals consuming these yeasts. When they form 30% of the pig ration (an excessive proportion since it already supplies 18 per cent of the crude protein content), a figure which rises to 24% if the cereals are allowed for in the ration), 90 ppm of n-paraffins have been found in adipose tissue.

For normal use-at the rate of 10-15%-the residual n-paraffins should be reduced by one-half or two-thirds.

In the U.S., an n-paraffin content of 2,000 ppm is permitted in bread and confectionary and 950 ppm in meat. In France, coffee may contain as much as 20,000 ppm! In Italy, "rice for boiling" and "superfine rice for soups" contain respectively 40 and 1,400 ppm of n-paraffins, and grissini between 20 and 80 ppm of hydrocarbons.

It is known that many hydrocarbons, including the paraffins, are synthesized by grassland plants and vegetables. The same process applies to fatty acids having an odd number of carbon atoms. Bandurski and Nagy (1975) have found fatty acids and C_{14} to C_{35} alkanes including the C_{17} in beef heart lipids. These compounds also occur in mammalian (including human) milk, and consequently in butter. One danger of the presence of such lipids is that they may be considered to be the same as all others when rations are being formulated. For example, the C_{17}:2 acid has nothing in common with the C_{18}:2 linoleic acid. We are making this observation because we have seen such mistakes made in designing experimental diets. All that was needed was to correct the lipid imbalance by using 1.5% of maize oil in the ration, thereby causing the disappearance of effects that used to be attributed to a deficiency of essential fatty acids.

Long-term studies on 17 generations of rats and 25 generations of quail prove that the lipid compounds of yeasts have no toxic effect. In any event, fatty acids having odd numbers of carbon atoms are eliminated by the normal lipid-replacement process, which takes one month. These fatty acids

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1. International Union of Pure and Applied Chemistry.

2. For the purpose of these rates the grains and vegetables mentioned earlier must therefore be considered separately.

3. It has been shown (Table II, footnote) that the polycyclic aromatic compound content of yeasts now in production is approaching the lowest measurable limits.

are metabolized at least as easily as the even-numbered-carbon fatty acids.*

It is because of the presence of such lipids that we have often been asked whether the so-called transmitted toxicity methodology should be applied to protein-producing microorganisms. We have always answered in the negative. Very lengthy testing, often covering several generations, seems sufficient to ensure a high degree of safety. There is no possibility that suspect elements clearly identified in analysis and known to cause the formation of unknown metabolites in the livestock animal could be hazardous to the human consumer-who, in any event, carefully eliminates the fat from any meat served to him. There is equal justification for carrying out transmitted toxicity tests on meat and offals from animals which have consumed as part of their rations those cereals which, as we have shown, may sometimes have a high content of benzo-pyrene.

The Effect of Technological Processes on the Toxicity of the End Product

In most cases, technological processes are used to eliminate the maximum amount of suspect products. Could these processes be responsible for possible toxicity?

A fermentation process is in itself liable to give rise to dangerous compounds. For example, amino acid decarboxylation phenomena give rise to toxic amines and polyamines[1]. Similar reactions occur in the belly of ruminants as a result of feeding an unduly high proportion of glucides, which depress the pH of the rumen. Such occurrences are not excluded in an industrial fermenter, just as the self-manufacture by the microorganism of toxins peculiar to it should not be excluded.

The use of organic solvents to remove lipids from protein-producing micro-organisms may cause toxic compounds to form. Reports of such occurrences alert us to this risk and strengthen our suspicions. Soybean meal from which lipids were removed by trichlorethylene have caused anaemia in bovine animals and nephrotoxic disorders in rats. Chick oedema, brought on by the presence of polychloro-dibenzo-p-dioxides in fats added to the ration and extracted by means of chlorine solvents, is a further example of the effects caused by the use in lipid extraction of certain organic solvents. The polychloro-dibenzo-p-dioxides act, in the case of poultry, at an intake of 9 µg/kg per day. Monkeys and pigs are also sensitive to these toxins (see Bibliography, under Anonymous).

The production by ammonia treatment of alkane yeasts with a low nucleic acid content causes potassium to be eliminated: the potassium level drops from 2% to 0.43 - 0.48%.

The use of yeasts whose nucleic acid content and potassium content has been lowered in this way causes a potassium deficiency in rats. Gatumel and Shacklady (1975) have proved this to be so. They point out that the addition of 0.2 ppm of selenium to rations containing 28% of alkane yeasts from the Lavéra plant (France) increases the weight gain of poultry consuming such rations. Selenium is also known to be effective in diets containing torula yeasts.

A knowledge of all the details of the various treatments used during the production of protein-rich microorganisms would be helpful in the toxicological and the nutritional researches. Coordination between specialists in these two fields would enable the effects due to toxic substances to be distinguished from those attributable to nutritional imbalances. The former can be eliminated and the latter corrected.

Changes or adaptations of the processes used are sometimes sufficient in themselves

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*cf. Garattini Report concerning these questions.

1. Ptomaines or the putrefaction "ptomaine" poisons of English-speaking writers.

to bring about a complete change in the nutritional value of the end product. For these reasons, it is essential to know all details of all stages of production and purification and always to verify whether products prepared industrially are identical with those produced and tested at the pilot stage.

Presence of Heavy Metals or Pesticides

Any analysis of the biomass enables the presence of either heavy metals or pesticides to be ascertained. Extremely precise techniques are currently available for this purpose.

Single-cell or multi-cell organisms possess the capacity to "fix" from the substrate certain valuable substances such as the B-complex vitamins as well as some harmful substances. Kokke and Kujala (1974), for example, have observed that yeasts can accumulate mercury and DDT. Yeasts are capable of anaerobic and aesobic degradation of DDT. There is some reason to think that the presence of polycyclic hydrocarbons, n-paraffins and amino acids with an odd number of carbon atoms is attributable at least in part to these mechanisms.

It is therefore important, as we said earlier, to know what impurities are present in the substrate in order to anticipate possible concentrations of them in the biomass. The more complex the substrate-and here we are thinking of liquid manures-the greater and more varied the risks of concentration scale.

PAG, like IUPAC, proposes in documents cited earlier maximum limits for metals not to be exceeded in a product having a moisture content of under 10. These limits are:

Lead	< 5 ppm
Arsenic	< 2 ppm
Mercury	< 0.1 ppm

Some thought should also be given to cadmium, perhaps to strontium and to pesticide residues other than DDT and its metabolites, or again to certain alkaloids, even hydrocyanic acid, when the substrates are potatoes, manioc or their by-products. This list is not exhaustive. Substrates and processes employing them must, depending on their nature, receive the attention of experts using their imagination, tempered by common sense.

Decomposition by Microorganisms

The question of decomposition by microorganisms is important, whether one is dealing with contamination in the course of the preparation of the biomass or with its purification or storage. The process is not, however, in any way different from contamination of conventional animal feeds. The results of analysis, for bacteriological purity, of industrially-prepared yeasts shows that the microbial pollution of these yeasts is much lower than that of fishmeal and soybean cake (Table III). In any event, limits have been suggested by PAG and IUPAC in the documents mentioned earlier. These limits are:

Microorganism	Number per gram
Viable bacteria	< 100,000
Viable yeasts and molds	< 100
Enterobacteriaceae	< 10
Salmonella	< 1 per 50 g
Staphylococcus aureus	< 1
Clostridia, total	< 1,000
Cl. perfringens	< 100
Lancefield Group D Streptococci	< 10,000

In our opinion, apart from mishaps in the fermentation process or contamination of the fermenters, pollution by microorganisms is much more likely to occur during storage and, still more, at the time of blending with other foods which are known to contain numerous microorganisms. Up to now there have been no regulations governing conventional animal feeds or the traditional yeasts. The case of fishmeal and meatmeal was

mentioned earlier. These are not the only ones. All the customary foodstuffs may be compared to their disadvantage with protein concentrates derived from microorganisms.

Conclusions

For the purpose of research on the toxicological aspects of SCPs, the experimenters should be given guidelines but not subjected to a set of rigid constraints. Those who try to prove too much often manage to prove nothing. One should avoid being influenced by fashion and by words. Nothing is easier than to raise the spectre of mutagenic effects in the proposed research. It should be recognized, however, as the specialists of WHO have done, that no reliable methods as yet exist for accurately measuring the existence and magnitude of these effects. Let us be reasonable and prudent in order to be more effective. The facts should be examined objectively and practically. The resources of imagination should be allowed to apprehend reality without being carried away by dreams. One must "pursue the ideal while understanding the real", as Jean Jaurès said.

To our knowledge, never in the case of any food product have so many precautions been taken and so many scientists been called upon to state their opinions. One of the authors recently observed, keeping in mind Beaumarchais' famous remark that the qualities demanded of unconventional foods are such that we know of few conventional foods which would deserve to be described as first-class.

We have mentioned the pitfalls which words and fashions represent. This applies equally to certain terms. They conceal dangerous potentialities capable of provoking unexpected reactions. By allowing the imagination free rein they can destroy all critical reasoning and all common sense. The word "petro-proteins", used by so many journalists and even by scientists is this kind of term. We don't talk about sulfite-proteins or molasses-proteins. Terms such as single-cell proteins, microorganism proteins and, better still, bioproteins would be more suitable and less disturbing.

It has always been important to check carefully the virtues and defects of these new sources of proteins in order to accentuate the former, mitigate the drawbacks of the latter and thus establish rules and limits for their use in animal feeding. In this way, the new nutritional balances they demand will be established, as will any possible harmful effects they might have-either by disturbing nutritional balance or owing to the presence of substances creating "health hazards". Requirements for the chemical and biological stability and integrity of new products during manufacture and storage and, if appropriate, subsequent processing, must also be studied carefully.

Our protein resources are small. The consequences of excessive urbanization at once create rising demand and reduced production. The use--at first for animal feeding--of microorganisms grown on a great variety of substrates, including grains and even certain waste matter, appears to us to be one of the most elegant solutions to this serious problem of our times. It is hoped that this paper will help provide guidance for adoption by warning against certain mistakes. We have tried to draw attention to the need never to overlook the practical use that will be made of these products in animal feeding. For these products, as for any food, toxicology and nutritional balance cannot be separated without risking a wrong interpretation of test results. Indeed, in the case of these novel protein sources, the relationship between toxicology and nutrition is even closer than in the case of a toxicological evaluation of additives alone.

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Table I.

Effect on Growth, Consumption Index, Protein Efficiency and Uraemia when 10, 20 and 30 per cent of CPC of the Ration is Supplied by One of Three Sources of Protein: Casein, Eggs, Alkane Yeasts

(9 Male Wistar Rats per Sample)

Source of proteins	Casein			Eggs, lyophilized and heated to 60°C			Alkane yeasts (Toprina)		
Theoretical percentage (CPC)	10	20	30	10	20	30	10	20	30
Real percentage (N x 6.25)	10.69	22.5	30.2	10.57	20.70	30.25	9.77	21.51	30.84
Initial average weight (g)	42.6	42.8	42.7	42.4	42.7	42.8	42.4	42.6	42.8
Final average weight (g)	112.7	121.9	114.1	119.3	111.3	122.3	105.6	121.8	120.7
Daily average gain (g)	2.50	2.82	2.55	2.74	2.45	2.84	2.25	2.82	2.78
Consumption index	3.74	3.35	3.58	3.23	3.56	2.86	4.04	3.39	3.50
Protein efficiency	2.49	1.32	0.92	2.84	1.35	1.15	2.44	1.36	0.92
Urea mg per 1000 ml and range of values	193 (190-200)	288 (205-355)	443 (410-466)	168 (140-190)	368 (305-410)	423 (385-490)	273 (250-350)	328 (325-335)	366 (360-380)

Table II.

Content (µg/kg of Dry Weight) of Polycyclic Aromatic Compounds in Certain Yeasts

	3:4-benzopyrene	1:12-benzo-perylene	1:2, 5:6-di-benzanthracene
Bakery yeasts "La Parisienne" origin: Epernon	12.2	4.3	0
Bakery yeast "St. Louis" origin: Marseilles	8.0	3.8	0
"Gayelord Hauser" diet super-yeast origin: Epernon	0	0	0
German bakery yeast "Deutsche Hefe" GmGH origin: Hamburg	13.2	9.7	0
German bakery yeast for diets "Deutsche Hefe" origin: Hamburg	0	0	0
Russian industrial yeast origin: "Le François" Paris	8.7	6.0	0
BP yeast, origin: Lavéra*	0.55	0	0.1
BP yeast, origin: Grangemouth*	2.5	1.3	0.5
ELF ERAP yeast origin: Lyon-Solaize	< 1, and same level for benzo-4-fluoranthene and 20-methylcholanthrene		

*These samples of yeast come from experimental fermenters. The yeasts currently in production do not show any traces of these compounds (see report of R. Ferrando and G. Bories), the detection level being 1 ppb (µg/kg).

Table III.

Bacteriological Purity: Comparative Results of Analyses of Toprina Yeast, a Fishmeal and Soybean Cake

(Expert's Documentation)

Organisms per g of sample	BP Yeasts	Fishmeal	Soya-cake	Methods
1. Aerobic bacteria count				
a) Mesophilic bacteria	910	50	23,000	Agar, tryptone + yeast extract at 30° C
b) Thermophilic bacteria	1,500	2,600	10,400	Same medium at 55° C
Total	2,410	2,650	33,400	
c) Thermophilic bacteria spores	60	145	890	Agar, tryptose + soluble starch at 55° C
d) Molds and yeasts	0	0	100	OGA agar at 20-22° C
e) Enterobacteriaceae	20	0	10	Mossel medium at 30° C
2. Coliform	0	0	0	Brilliant green-bile lactose broth
E. coli	0	0	0	MacKenzie test
3. Salmonella Arizona	0			Selenite-tetrathionate enrichment broth, then DCL and Kligler
4. Staphyloccus enterotoxins	0	0	0	Baird-Parker medium, coagulase test
5. Sulfite-reducing Clostridium				
a) Cl. perfringens enterotoxins	0	2	10	Results after 18 hours
b) Cl. perfringens	2	2	85	
Total sulfite-reducing	2	2	190	
Total sulfite-reducing	10	20		Results after 48 hours

REGULATORY ASPECTS OF SCP FOR ANIMAL FEEDING

The Experience in France by G. Bories*

Various countries are now considering setting up registration protocols for non-conventional proteins in order to satisfy their own requirements with respect to public health. Even if they do not yet have their own production capability, they may be affected by community trade within the EEC or by international trade and must therefore have the same security and safety rules governing the use of those products.

The problem arises rather differently in France. In 1969, the French government was requested to register industrial production methods for yeast grown on gas oil.

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In view of the fact that the problem was very novel, a great deal of work was undertaken to set up an approval protocol as well as a control procedure. The French position concerning the use of non-conventional proteins was hammered out on the basis of that concrete experience. At that time, PAG's proposed Guidelines reflected similar concerns and concepts. Since then, work was undertaken to set up a European directive which was largely inspired by the two earlier approaches. The purpose here is merely to relate the experience acquired in France over the last 5 years.

The French position concerning the use of non-conventional protein sources is based on various ideas. In particular, the industrial production of bioproteins uses an entirely new technology. On the one hand, this is due to the fermentable substrates used and also due to the size of the production units implying a considerable extrapolation of microbial ecology expertise. Other salient features of the new technology include the automatic control of production units and the sterilization of cultures. The French authorities, seeking to guarantee public health and well aware of the psychological impact of such a development on consumers, decided to reserve the use of these products for animal feeds; secondly, it was determined to submit these products to various tests meant to show their safety and their nutritional value for animals; thirdly, assuming that these measures have been taken, controls should ensure that the production of each plant conforms with the standards set. In all events, these new proteins could not be considered simply as feeds, such as conventionally produced brewers' yeast or bakers' yeast.

The study protocol was set up above all to ensure the hygienic quality and nutritional value of these products. With regard to hygienic quality, the size of the risk taken in the case of SCP is related to the purpose of their destination. Administration to animals may give rise to direct risks to the animal and perhaps also to an indirect risk for the human consumer, however attenuated. The second phase, the direct consumption by man, would require greater precautions, but would also in turn benefit from the experience acquired with animals.

The potential risks inherent in the characteristics of the substrate and microorganism have been mentioned here on several occasions.

The actual risks can be assessed in connection with well-known and defined substances (such as heavy metals or polycyclic aromatic hydrocarbons) by reference to the available toxicological documents and by a very strict analytical check in the finished products. As far as the undefined substances are concerned (the antinutrient substances and toxins which may exist in such products) these can be defined by deeper toxicity studies. Toxicological experiments must always take into account the nutritional requirements of each species concerned, as well as the nutritional balance of the diets.

In France, there are separate approval and control procedures: the procedure for authorization is based on the examination of files presented by applicants and is based on the protocol mentioned earlier. The Inter-Ministerial and Inter-Professional Commission for Animal Feeds then examines the files. It will also draw upon the work done by its restricted committee which also ensures that controls are properly organized.

The control procedure is then set up by the restricted committee in the shape of a test protocol, based on data from the files and adapted to the special case of the product under consideration, the nature of the substrate and the production or marketing conditions. Of course, different protocols will apply to products manufactured in the country itself and those that are imported.

For proteins manufactured in France, the firm must assure a permanent control of its production; the official laboratories, which have free access at any time to the analytical data gathered by the company, carry out super-control with a frequency

compatible with their needs and responsibilities. Information is circulated between the official laboratories which carry out various assays and the controls carried out by the company.

Under France's procedures for marketing gas-oil grown yeast, the granting of authorization and the setting up of controls were carried out according to the following pattern: the control or test protocol for gas-oil grown yeast stipulated a) the spirit of this check; b) the nature of this check and distribution of work between the various official laboratories; c) two phases of sampling conditions - the first, to set up the production unit, and the second, the operating phase of the plant; d) guidelines for how a sample should be taken, identified, distributed, and how the results should be collected.

Over 3 years of checking, a total 295 samples were taken, corresponding to 45 batches of yeast and a sample of 3,600 tons of products. 1,086 analyses were made, 320 microbiological controls, 228 controls on residual hydrocarbons, 111 tests of residual isopropyl alcohols, and 34 organic tests. A total of 15 analyses of amino acids were made along with 20 studies of protein efficiency, 11 analyses concerning mineral compounds, 16 analyses of trace metals, 22 analyses for lead and arsenic and 15 analyses for 3,4-benzo-pyrene. The results of these checks allowed us to see, first, that all the samples checked were in conformity with standards existing at the time authorization was given - e.g., the amounts of protein, residual hydrocarbons and residual isopropyl alcohol. Horizontal checks fixed 0.08% for residual hydrocarbons in gas-oil grown yeast and at least 66% proteins in yeast.

As to microbiological test results, Professor Ferrando reported that in every case the level of bacterial contamination was considerably below the standards set by PAG and were, in comparison with other types of animal feeds in current use, at the same or often much below their level. The amounts of benzo-pyrene have always been below or equal to 1 ppb.

The next step concerned the dovetailing of results obtained by the company's test laboratory on the product when ready for consumption with those of official laboratories. Here again, we observed the same type of distribution of the results as during the 2 1/2 years during which tests were run.

These satisfactory results were made possible by the cooperation between the company concerned and official services. It is obvious that the objectives of the two parties were identical during that first phase when a new technology was being set up. Because the psychological impact of such technology is decisive, a dialogue was established between the laboratories, making it possible to make progress in control methods and techniques, as in the setting up of a protocol for microbiological tests (fermentation conditions, review of standards). Then there was also a comparative study of two different methods of analyzing the residual hydrocarbons, one by infra-red spectrometry producing automatic checks during manufacture and the other by a gaseous phase chromatography adopted as the official reference method.

The Experience in Italy by L. Bellani*

From the legal point of view, the problem of manufacturing, distributing and using n-alkane-grown bioproteins arose in Italy for the first time in 1971. Existing legislation entrusts technical research to the Health Council of the Ministry for Public Health which must examine the dossier produced by the company. Because the subject was such a novel one, however, the Ministry ordered a preliminary examination by the Higher Public Health Institute, the Ministry's scientific organ, as well as by a special group of experts nominated by the Council. Following the rendering of a

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favorable opinion, the whole matter was discussed by the Council which is the highest body dealing with public-health matters in Italy. The Council, while fully aware of the novel nature of the undertaking, passed favorably on the production, marketing and application of this type of n-alkane-grown protein. The Minister for Public Health, together with the Ministers of Industry and Commerce, and Agriculture, signed the authorizing decree in November 1972.

It should be noted that the decree classified the bioprotein as an industrial chemical product. For such products, it was sufficient to have it registered in the official list. This provided authorization not so much for the production, but rather for the marketing and consumption of the product in the future. Nonetheless, following the recommendation of the Health Council, the Ministry gave its authorization, and at the same time set up plans to undertake a considerable research program with two aims in mind: the first was to check on the various tests; for Italy, this was knowledge which had been imported from abroad as it were. Secondly, it was deemed necessary to specify and conduct certain other tests, not because of any doubt as to the efficiency or safety of the product concerned but rather due to problems which arose from the complex production technology. It was thus proposed to study the stability of the strain in biomass production; and to test the validity of the statement that there were no residues in the tissues of animals which had been fed those substances.

Meanwhile in 1971, another Italian company had requested an authorization. This, however, was stopped immediately because there were some doubts concerning the pathogenic effects of the strain used in fermentation. In 1973, the firm - which had been authorized to produce, sell and use that substance - made a further request to change the composition of the product. Above all, authorization was asked to increase the permitted levels of certain heavy metals. By then the Ministry of Health was able to have a new look at the entire problem in the light of developments in the technology and knowledge gained as a result.

The first item now of concern to the Ministry and to scientists in Italy was the question of the health and the sanitary and hygienic practices in the plant and its environment; second, possible pathogenic factors in the strain, and third, the problem of residues in the foods obtained from animals which had been fed those substances. The discussions, which began in 1972, involved the various bodies mentioned earlier. All this gave rise to a second decree, published in February 1974, in which the Ministry of Health, with the agreement of the Ministries for Industry and Agriculture, took the position that it was possible to manufacture, market and use the n-alkane-grown yeast in animal feeds under the following conditions:

1. The plant should have to be set up and be operated under classic hygienic conditions.

2. Hygienic precautions would be checked in the course of production, and notably, before the goods were marketed. The decree stipulated that this was to be done by the Health Institute.

3. The production should utilize all appropriate safeguards for the staff employed.

4. The use of the bioprotein by animal feed producers should be controlled. There should also be controls instituted for the use of these substances in cattle farms.

The decree included two interesting variations, the first requested by the enterprise itself that the tolerance limit for heavy metals should be changed. The second variation was in the microbial content that could be tolerated in the finished product. It is sometimes said that production should be carried out under conditions of complete sterility. This, however, is impossible since the fermentation processes cannot be carried out under conditions of absolute sterility - there must be a certain tolerance. Thus, it was determined to control all pathogenic microorganisms and to set limits on

the non-pathogenic microorganisms that could be present. Meanwhile, experiments begun in 1972, continued to be conducted in the research bodies of the Ministry for Agriculture, the Zootechnical Institute in Rome and at other institutes and universities.

During the first research phase, the studies instituted had produced very positive results. The Italian experiments made it possible to extend the product's use in certain types of animals not contained in the first decree (only broiler chickens, pigs and calves were contained in the first decree). It was found that this product could also be used for the feeding of other animals - milking cows and goats, as well as for fish. The results relating to performance - within the percentage limits set forth in the decree - were entirely satisfactory.

The second experimental phase dealt with studies of tissue residues of animals fed the product. Because of the high content of nucleoproteins in SCP one had to see to what extent nucleoproteins could vary in animal tissues destined for human consumption. The other constituents studied were the percentage of odd fatty acids and residues due to the n-paraffin or n-alkane substrate in tissues of yeast-fed animals.

The results were rather surprising. The amount of nucleoproteins varied; the odd fatty acids appeared in larger quantities in animal tissue and high quantities of n-paraffin were present in the fatty tissue of pigs. The Ministry of Health asked research workers to review the effects of these observations on human health. One can understand why the discussions took so long. There was great difficulty in calculating the risk to the population, risk due to the presence of certain elements in the tissues of animals destined for human consumption. The Institute stated in July 1975 that they were unable to give an opinion on any of these questions and they asked that research be continued. In particular the Institute recommended that the Health Council be convened to review the whole problem along with the physicians. A debate in July 1975 concluded that these three residues gave rise to doubts regarding the absence of risk for animal and human consumption. The authorities therefore advised that research be continued in order to find out precisely what these risks were and what one could do against existing drawbacks. They advised that, for the time being, no authorization be granted.

Many people within the Health Council had asked that the decree be withdrawn or cancelled. In July 1975, the Council transmitted its views to the Ministry of Health and, in February 1976, the Ministry, together with the Ministry for Industries, suspended the decree of 1974 and allowed production only for experimental purposes. The explanatory memorandum states that the main concern stems from the presence of n-paraffin residues, whereas the raising of nucleoprotein and odd-numbered fatty acid levels was not deemed to endanger public health. In the present state of affairs, the production, marketing and consumption of bioproteins in Italy is no longer allowed and therefore it is no longer possible to import these substances except for experimental purposes. Further, it is no longer possible to import - either from EEC countries or from third countries - any animals which have been raised on alkane-grown yeast.

The legal position merits further study. Professor Garratini has shown that experiments are being carried out in Italy which attempt to find a solution within the toxic, hygienic and health framework of animal feeds. This, of course, applies to n-alkane-grown yeast meal.

These problems are difficult ones. On the one hand we must not be carried away by non-scientific or extra-scientific considerations. On the other, governmental decisions in areas such as this should not always solely be influenced by a scientific report here or there. Public opinion frequently looks at scientific discourses with suspicion. The public raises objections throughout the world on the manner in which the world uses its

resources. Governments cannot, of course, afford to disregard what is being said. We remember too well what went on in Japan; therefore, what happened in Italy is not really so surprising. We must not underestimate the importance of public opinion in such areas.

In other words, this is a matter of conviction and participation. Conviction and participation can only be obtained if sufficient amounts of information are available from scientists. One must not be surprised if people disagree, not only on the interpretation of the presence of residues, but also regarding certain experimental methods themselves. Some of the research findings have emanated from the research laboratories of the industries engaged in SCP development, as well as from the chemical and biochemical industries. While their work is useful, the greater participation of hygienists, biologists, cancer specialists and physicians is necessary in arriving at conclusions.

These professional groups, when faced with a problem of this type, must overcome the knowledge gap, especially since their interests have not been linked to these subjects. Similarly, discussions on metabolic disorders and carcinogens in food should not be reserved to food biologists. These are subjects in which other scientists must participate as discussants. That is why meetings such as this one should not leave the problem to the vanguard. We must have not only the cooperation of these eminent personalities but also that of institutes which are particularly competent in tumors.

In conclusion, I would emphasize, we require the participation of the scientific world with a wide range of expertise. The citizens must also be well informed. I am here in order to support the decision taken by my government and to remind you that one must be extremely careful in fields such as this. We are still awaiting the decisions to be taken by the EEC Market and also want clearer explanations from PAG on the various issues.

The Experience in the Netherlands by W. Wiegaraadt*

The way in which animal feed legislation is effected in the Netherlands differs considerably with that of the other EEC countries, where the central government is in charge of the legislation. In the Netherlands, however, the central government has delegated legislative competency to the Commodity Board for Animal Feed.

The board of this public organization is composed of representatives of different branches of trade and industry that deal with animal feed and raw materials for the production of compound feedstuffs; it also includes farmers and representatives of the pertinent ministries.

The central government has not conferred complete independence on the Commodity Board. In order to protect the public interest, all Commodity Board regulations must be approved by the ministers involved. Further, the central government has retained the right to pursue its own initiatives within the trade and industrial sectors.

It should be clear that in such a situation where trade and industry are directly involved in legislation, there is no call for regulations for which there is no need in practice. Thus, the regulations of the Commodity Board aim mainly at ensuring that those animal feedstuffs that are delivered to the farmer do not harm indirectly the health of the consumer of animal products.

In this sytem restrictions on the raw materials destined for use in the manufacture of animal feedstuffs is made only in exceptional cases. So far regulations have been invoked only in the cases of fats, oils and wheat offals, due to HCB residues; groundnut products, because of Aflatoxin B_1; and

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animal proteins in relation to Salmonellae. Further, the use of dried poultry-manure as a component of compound feedstuffs has been prohibited.

This approach has always worked satisfactorily in the Netherlands insofar as the traditional prime materials are concerned. Thus, when several companies some years ago initiated the production of SCPs on the basis of hydrocarbons, there was no reason in the first instance for the Dutch authorities to impose restrictions since these products were being developed as prime materials and not as straight feeds. In time, however, this point of view was changed because it had become clear that some products posed potential risks to the health of man and animals. The Dutch Commission, which among other things oversees the introduction of additives to animal feedstuffs carefully followed the development of the SCPs mentioned.

Two reasons are apparent to explain the current absence of legislation in the field of SCPs in Holland; SCPs are not yet incorporated in animal feedstuffs on a commercial scale in the Netherlands; secondly, the European Commission is itself working on a proposal for a directive and is drawing-up guidelines for testing the concerned products.

It would appear possible to link closely the European Commission's guidelines with those drawn-up by PAG. In any case, parties should avoid the fear that appears to exist for developments in the manufacturing of non-conventional prime material for the production of compound feedstuffs. Such fear results in unnecessary discrimination in relation to the traditional raw materials used for such feedstuffs.

The Experience in the U.K. by A.G. Ward*

The whole subject of novel proteins, including some consideration of their use as animal feeding stuff, was reported in the U.K. Food Standards Committee Report (F.S.C.) on novel protein foods. Paragraphs 57 and 58 in particular raise the question of the use of these products for animal feeds. The consequences for the resulting human food is given, with a little detail about the discussion. Consideration was also based on a report made by an official committee to the FSC on the toxicological aspects; our conclusions in the FSC rested in part on that advice. As far as that report went, the conslusions were that no recommendations for regulation were deemed necessary at that time.

The spirit of the legal approach in the U.K. is fairly parallel to that within the Netherlands, where the attitude is similar, although the actual application is somewhat different. The clearest issues arise in relation to the actual effect on the animal itself, its performance, and its health, of the use of SCP in animal feeds. These provisions are covered by the Agriculture Act of 1970; there is here no special reference to SCP since its control is the same as the control exerted over any other component in a feedstuff.

The points to which attention should be drawn are, first, the need to give adequate information to the purchaser. Second, the sale of a feedstuff implies a guarantee of its suitability, at least for the uses for which it is proposed. Third, it must not contain substances harmful to the animals for which its use is intended, including substances which are toxic or which may in some other way harm metabolism, growth and other health factors in the animals. In animal feedstuffs, as in human food, the final decisions rest in the courts and not with administrative decisions in ministries.

The operating companies interested in this area in the U.K. have extensive research programs and are in close consultation with the Ministry officials concerned with feedstuffs and the law affecting them. It is

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in the interest of those companies as well as of the government to ensure that three things are done: The first is to see that the law as it stands is obeyed. Secondly, ensuring that experimental work is carried out as far as possible to prevent any unforeseen harmful effects arising. Finally there remains the clear commercial implication of any failure in this area, since this would set back the program of development of SCP and its utilization very considerably. Left to itself, it is relatively unlikely that the U.K. government would draw up any detailed regulations in this area. The provisions of the Act as it stands are seen as sufficient to lead to prosecution since it is an offense if any of the obvious breakdowns in action by an industrial company occurred. So while the U.K. government will probably not take any further action, it will participate in possible EEC developments that may arise or that are indeed already under consideration, although it may attempt to limit the extent of regulation and restraint in this area.

In relation to the food which results from feeding SCP to animals, countries like the U.K. and the whole of Western Europe have a diet such that, in terms of physiological need, additional protein is not required. So the objective of making it possible either to maintain or increase animal stocks and animal production in order that meat intake can be maintained is not basically a nutritional requirement but rather a matter of demand to eat that particular form of food. It is no less compelling an objective for this, but one should be clear what the reasons are. This means that any U.K. government interest in the meat, milk or eggs that come from animals fed SCP will be confined purely to safety aspects.

The current view in this connection is that there is no clear indication of sufficient risk to call for action unless this arises from negotiations in connection with an EEC directive. In this area also there is already the protection of an Act of Parliament, the Food and Drugs Act of 1955, which provides safeguards to the extent that any meat, milk or eggs causing harm to health as a result of the animal having been fed on SCP will constitute almost certainly an offense under Section 1 of the Food and Drugs Act. If the food is changed significantly from its previous form by the new form of feeding - which is again extremely unlikely - an offense is committed under Section 2 of that Act (that is, the section which says that a food must be of the nature, substance and quality demanded by the consumer). Of course, the problem of what is implied in this is a matter for the courts, and not again for administrative action. Direct consumption of SCP by humans is, of course, a different matter and one for which the question of control will certainly require a different approach.

Finally, I just want to mention briefly the Food Additives and Contaminants Committee's recent report on the subject of hydrocarbons in food. Although the report of that committee does not carry the force of law, I think it is likely that regulations will follow a report of the committee concerning this subject. In relation to the use of SCP for animal feed, the conclusion of that committee is that regulatory action is unnecessary here unless evidence of carryover into the meat of such hydrocarbons from the substrates is significant. With regard to the direct use of such material for human food they express the view that an early warning will be needed because once again it will have to be looked at in much more detail.

Summing up the situation in the U.K., the position is that there are Acts of Parliament, two in particular - the Agriculture Act of 1970 and the Food and Drugs Act of 1955 - whose general powers appear presently adequate to exercise control in this area. The danger of detailed regulations in an area of this sort is that often these may be framed in ways that might impede the progress of discovery, development and industrial application in areas which do not contribute to the nutrition or growth of animals, nor to the human food

which they can contribute. I would conclude by putting the plea for caution in terms of the extent of regulation in this area, unless and until it becomes crystal clear that such regulation is necessary.

STATEMENT MADE BY REPRESENTATIVE OF EUROPEAN ECONOMIC COMMUNITY*

Since 1973, SCP has given rise to special interest as a possible source of proteins for the EEC. Needless to say, the EEC depends to a large extent on other countries to meet its protein needs for animal feeds. During the last 5-10 years, the EEC has been able to supply from its own resources only about 20% of its protein need. The protein requirements are mainly covered by one product - soya, produced only in a few countries around the world. This may be either an advantage or a drawback. After the 1973 crisis, certain hopes arose regarding SCPs as a protein source. However, some important phehomena have occurred since, causing these hopes not to be fulfilled. The first factor was the rise in the price of petroleum, the raw material used for the production of SCP. On the other hand, we have encountered a drop in the market price of soya since 1974. Thus, the cost of SCP follows certain industrial development trends, whereas these products are meant as substitutes for agricultural products that undergo sharp price fluctuations in the world market. In other words, the market reacts very suddenly and elastically to prices because the world's overall requirements for protein are not elastic. Since there is no expectation of any great change in the present position, the economic conditions for producing SCPs in the EEC are not very encouraging, to say the least.

The difficulties in which the SCP industry finds itself have not prevented the EEC from undertaking preparatory work regarding legislation on SCPs. The basis of this work was laid down in a resolution of the Committee of Ministers on 22 July 1974, which provides that the Council should be led to adopt legislation concerning bioproteins. On the basis of this resolution, preparatory work was started so as to regulate the circulation of these products within the Community. Since these products have no tradition in animal feeds, work on this regulation is not proceeding as quickly as hoped. The EEC wishes to settle the circulation or use of certain oil cake products and other protein sources such as urea. At present, the draft Council directive is expected to contain two annexes. The first deals with those products circulating freely throughout the EEC, while Annex 2 deals with products that have a restricted circulation. We hope that we will be able to submit this directive to the Council of Ministers in the autumn of 1976. It is planned to modify the annexes if necessary when the technology and scientific knowledge develops further. The method planned for putting new products into the Annex is based to a large extent on the Guidelines adopted by PAG. Therefore, it is expected that a new product can be put in Annex 1 only if it is incorporated in animal feeds, has a favorable effect on animal production and has no unfavorable effect on animal or human health. In other words, our work is still in a preparatory stage and can benefit greatly from events such as this symposium.

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*Statement made by Mr. H.H. Wachter, Head, Organization of Markets in Crop Products (Agriculture), Commission of the European Economic Community, Brussels, Belgium.

MICROORGANISMS IN DAIRY PRODUCTS -- FRIENDS AND FOES

Barbara P. Keogh*

Microorganisms are endowed with many enzymes which are essential for their own metabolic processes and which give them the ability to act either beneficially as necessary agents in the manufacture of certain foods or adversely as food spoilage organisms. Their ability to act in these roles varies according to the species and the food, the components of which are substrates for the enzymes. The role of microorganisms in the production of dairy foods will be considered in both of these contexts.

There are three main classes of enzymes which play a part both in dairy processing and in the spoilage of dairy products: (i) the lactases which are involved in the fermentation of lactose; (ii) the proteases which are involved in the breakdown of milk proteins to peptides and amino acids, and (iii) the lipases which are involved in the breakdown of milk fat to fatty acids. These enzymes may operate advantageously or disadvantageously. The ability of microorganisms to bring about these chemical reactions is utilized to advantage in the production of fermented milks and cheeses, while the breakdown of milk constituents by any of the three types of enzymes mentioned above is undesirable in the manufacture of products such as butter or milk powder.

History

For many centuries soured milk has formed a vital part of the human diet. Its use as a food was mentioned in the Bible (Deuteronomy XXXII, 14: Genesis XVIII, 8) and it was, and still is, known to the nomads of Asia Minor as part of their natural diet. It is known to have been enjoyed by the early Egyptians, Greeks and Romans and it is said that the early Romans taught cheese-making techniques in the countries they conquered. The famous Emmentaler cheese industry of Switzerland is believed to have started in this way. While the transformation of milk in flavor and texture to another very palatable food was utilized, the fact that it was the result of bacterial activity was not recognized until the time of Louis Pasteur.

In 1907 Metchnikoff, a Russian academic who worked at the Pasteur Institute, published in his book 'Prolongation of Life', theories on the life-giving properties of the Bulgarian fermented milk, yogurt. He believed that its consumption provided a defense against infection and the production of toxins by bacteria in the intestine. To it he attributed the good health and longevity of the Turks and Bulgars. Although his theories have since been discounted, yogurt is a very nutritious food which is easily digestible. Despite the primitive state of bacteriology at that time, his discoveries concerning the bacterial flora of yogurt have not, however, been discounted.

Metchnikoff attributed the special characteristics of 'Yahourth' to the activity of the 'Bulgarian bacillus' which was always present in it. His description suggests that this organism is our modern Lactobacillus bulgaricus. He noted also that 'a para lactic bacillus' was always present. This organism appears synonymous with Streptococcus thermophilus of today. It has now been established (Pette and Lolkema 1950a) that in fact the best flavored yogurt resulted from the associated growth of Lactobacillus bulgaricus and Streptococcus thermophilus.

There are many other types of fermented milks. Practically all nations have one or

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more such milk traditionally made by allowing the milk of donkeys, goats, sheep or cows to sour by the action of the lactic acid bacteria which occur naturally in the milk. Dahi of India, leber of the Bedouins, matzoon of Armenia, piner of Lapland are but a few examples. Today, many of these products are made commercially in countries far beyond their places of origin. Probably the best known to the Western World are yogurt, Kefir, Koumiss, acidophilus milk and some modern variations of these such as the fermented milk of Japan known as Yakult. There are too many to describe in detail, but the role played by microorganisms in the better known ones may be of interest.

Fermented milks

Yogurt

In the Middle East and southern European countries yogurt is still prepared for local use from the milk of buffaloes, cows, donkeys, sheep and goats, but the yogurt of the Western World is made from cows' milk. The name of the product has many spellings: yoghurt, yoghaurt, yoghourt, yahourth, yogurt, yaaurt, jugurt, yaert, yaoert.

The manufacture of yogurt in the Western World is scientifically controlled and is a more sophisticated process than that which produces the yogurt of the Balkans. There, the bacteria naturally occurring in the milk were used as the souring agents. In yogurt made commercially, milk is homogenized and heated to 85-90° C, held for 15-30 min, cooled to 45° and inoculated with a mixture of L. bulgaricus and S. thermophilus. These organisms are propagated either together or separately until inoculated into milk for yogurt manufacture. Incubation is at 43°. Heating the milk before inoculation not only destroys undesirable bacteria, but also drives off the oxygen thereby creating the right conditions for the growth of L. bulgaricus. Moreover, heating produces growth factors by denaturing the milk proteins. It has now been established that the L. bulgaricus and S. thermophilus stimulate each other (symbiosis). The S. thermophilus produces formic acid which is required as a growth factor by L. bulgaricus (Galesloot et al. 1968; Veringa et al. 1968). The L. bulgaricus with a higher proteolytic capacity, liberates essential amino acids, valine in particular, for the S. thermophilus (Pette and Lolkema 1950b).

During the growth of yogurt bacteria in milk lactic acid, diacetyl and acetaldehyde are produced by the fermentation of lactose and the breakdown of proteins. All these substances contribute in some degree to the flavor and odor of the product, but the most important is acetaldehyde which is produced by L. bulgaricus. The selection of strains for the production of these flavor compounds does not appear to be of much importance, but the viscosity of the product depends on the ability of the strains of L. bulgaricus and S. thermophilus to produce a polysaccharide from galactose (lactose $\rightarrow$ glucose + galactose) which acts as a thickener. Viscosity plays an important part in the organoleptic quality of the yogurt. Selection of strains on the basis of their ability to produce the thickener can eliminate the undesirable influence of seasonal variation in the milk (Veringa 1973) and obviate the need to use stabilizers such as alginates and gelatin which detract somewhat from the quality of the product.

Provided the conditions of manufacture of yogurt are strictly controlled, the product is not very susceptible to bacterial spoilage. The high degree of acidity precludes the growth of pathogens, but spoilage can arise from the growth of yeasts and molds. The lactose-fermenting yeasts, e.g. Torula cremoris, are the most common contaminants of natural yogurts made without added sugar, but yogurt production today has expanded into new styles with added fruits and flavorings, and in these the risk of yeast and mold contamination is increased. Trouble also arises from types of yeasts other than those which ferment lactose. Yeast contamination can lead to abundant gas production in the body of the product, while molds, being aerobic, form colonies on the surface. With both

types of contamination there is a consequent deterioration in flavor. Obtaining infection-free cultured products is a difficult task. Yeast and mold contamination from air and equipment can be avoided by strict attention to hygiene at all stages of manufacture, and contamination from fruit and syrup is reduced by the use of good quality raw materials which are adequately heat-treated before being added to the product. Fresh and frozen fruit give the best product organoleptically, but they are more liable to yeast and mold contamination.

Kefir

After yogurt, Kefir, is possibly the best known of the fermented milks. It originated in the Caucasian mountains and until recently did not move far beyond that region. Today, Kefir is popular in many countries of Europe and other parts of the world but by far the greatest commercial production of Kefir is in Russia. In 1964 one factory in Moscow was reported to produce 100,000 kg per day (Lang and Lang 1973).

The distinctive characteristic of Kefir is the development of granules up to the size of a walnut during its production. These are composed of denatured protein containing many different strains of yeasts and species of bacteria. The characteristics of Kefir result from the combined fermentation of the milk by lactic acid bacteria and by yeasts which produce about 0.8% lactic acid, about 1% alcohol and carbon dioxide. A good Kefir foams and effervesces like beer.

After fermentation the granules are filtered off and may be used immediately to inoculate new batches of milk or may be washed and dried and stored until required. When the dried granules are inoculated into milk, they rehydrate and once more start to ferment. The dried granules are commercially available in Europe but only to a limited extent in Australia because of quarantine regulations.

The fermented milk is a very refreshing drink. In some European countries fruit-flavored Kefir is popular.

Koumiss

Koumiss is very similar to Kefir, being mildly alcoholic and effervescent. It is traditionally made from mares' milk and originated in the Asiatic Steppes. Today in Russia, it is manufactured in large quantities from cows' milk and therefore differs little from Kefir. Currently it enjoys a reputation comparable with that of yogurt at the time of Metchnikoff. Thus it is said to cure many ailments and is believed to be especially rich in nutrients.

The starter organisms for its commercial manufacture are L. bulgaricus, L. acidophilus, and the lactose-fermenting yeast Saccharomyces lactis.

The special qualities of Lactobacillus acidophilus

As it has been shown that Lactobacillus acidophilus may become established in the human intestine, milk fermented by this organism has achieved some recognition in the medical field. Not all strains of L. acidophilus can establish themselves in the intestine, and among the variants which have this capacity others arise which do not (Anon. 1974). The therapeutic properties of L. acidophilus have been studied in Russia for many years, and as early as 1930 strains were isolated which produced acid freely and which were claimed to possess therapeutic properties. It was subsequently found that L. acidophilus produced substances which suppressed Escherichia coli and pathogenic intestinal bacteria. Strains of L. acidophilus were later selected on this basis (Lang and Lang 1973).

Fermented milks containing L. acidophilus have some place in the treatment of intestinal disorders resulting from antibiotic treatment. It is common experience that as a result of antibiotic treatment an imbalance of the natural intestinal flora occurs. Selected strains of L. acidophilus are apparently able to restore this balance. In England, an antibiotic-resistant strain of L. acidophilus has been isolated and is marketed for consumption during the course of antibiotic treatment,

either as yogurt which has been inoculated with the organism or as a dried powder for addition to milk.

Acidophilus milk or 'reform yogurt' which contains only L. acidophilus, and Bioghurt, which contains L. acidophilus and Streptococcus taette (a variant of S. lactis), have been consumed in Europe and the United States for many years. Acidophilus milk is a very acid product and many attempts have been made to develop a product with a better flavor. In some instances this is done by inoculating a quantity of milk with a mixture of S. Cremoris (or S. lactis) and an aroma organism such as S. diacetilactis or a leuconostoc or alternatively with L. bulgaricus and S. thermophilus, and mixing this fermented milk with another quantity of milk which has been inoculated with L. acidophilus.

In the product known as Aco yogurt a freeze-dried acidophilus powder is added to the milk at the same time as the normal yogurt bacteria. Another variation is a product called 'Acidophilin' which is made by fermenting milk with a mixture of L. acidophilus, S. lactis, and a Kefir culture. Yet another modification is 'acidophilus-yeast milk' which is made by fermenting milk with L. acidophilus and a lactose-fermenting yeast.

In Japan, cultured milk drinks are very popular and are regarded as having health-giving properties. They frequently contain autolyzed Chlorellae or Scenedesmus (algae) which stimulate the lactobacilli and extend their viability.

Use of aroma-producing bacteria

In cultured buttermilk and cultured cream, which are popular in Europe, the United States and Australia, the fermentative organism is S. diacetilactis which produces diacetyl. This imparts a butter flavor to the product. Butter manufacture in Europe also has a fermentation step in the process of manufacture: before the cream is transformed to butter it is fermented with a mixed starter containing S. diacetilactis and leuconostocs. Both these bacteria produce the diacetyl required for the enhancement of the butter flavor. Similarly, aroma bacteria may be used in cheese manufacture to obtain a buttery flavor.

Microorganisms in cheese manufacture

Lactic acid bacteria play an essential role in cheese manufacture. The starter cultures may be mixtures of S. lactis, S. cremoris, S. diacetilactis and leuconostocs or may be single strains of S. lactis or S. cremoris, the latter being generally employed in Australia and New Zealand for the manufacture of Cheddar cheese.

A common problem encountered in Cheddar cheese manufacture is the failure of the starter organisms to produce the desired acidity in the milk because starter organisms are susceptible to bacteriophage (bacterial virus) infection. While all bacteria are susceptible to bacteriophage infections, the problem is highlighted in Cheddar cheese manufacture because of the need to produce a higher degree of acidity in a relatively shorter time than in the manufacture of other cheese varieties. If cheese starters are selected on the basis of bacteriophage sensitivity patterns, the risk of infection is reduced and this is the reason behind the choice of single strains for Cheddar cheese manufacture in Australia and New Zealand.

In all types of cheese, from the soft cheeses such as cottage cheese to the hardest cheeses such as Romano, the lactic acid produced by the starter is an important factor involved in the removal of water from the milk. In manufacturing most cheese varieties, rennet is also added to help achieve this end. The desired amount of water to retain in the curd depends on the type of cheese being manufactured; it can be controlled by changing the temperature of the curd during manufacture as well as by adjusting the amount of salt and the method of its addition to the curd. The initial step in the manufacture of all cheese varieties involves a lactic acid fermentation by either the lactic streptococci or the lactobacilli, or both. In addition, other microorganisms may be used to achieve

special flavors and characteristics.

Under some conditions of manufacture certain strains of the lactic streptococci can bring about an undesirable bitter flavor, particularly in Cheddar cheese. This flavor arises from the bitter peptides produced by the action on the milk protein of the proteolytic enzymes of these strains of bacteria.

The aroma-producing bacteria, i.e. S. diacetilactis and leuconostocs are essential to the manufacture of cheeses such as Gouda and Edam, as well as many others where the buttery flavor of diacetyl is a characteristic of the variety. The aroma-producing starters also produce CO_2 which is responsible for small gas holes that are another characteristic of these varieties but are regarded as defects in Cheddar cheese.

Propionibacterium shermanii plays an important role in the manufacture of Swiss-type cheeses such as Emmentaler because it produces propionic acid and CO_2. The former is responsible for the slightly sweet flavor of the cheese and the CO_2 for the large gas holes. The size of the gas holes is controlled by carefully controlling the time the formed curd is in the brine used for salting and the maturation temperatures.

Other microorganisms are involved in the production of surface-ripened cheeses where a slime consisting of salt-resistant yeasts and Brevibacterium linens forms on the surface and produces enzymes which diffuse into the cheese. These bring about a very strong characteristic flavor from the breakdown of proteins and milk fats. Limburger and Tilsit are examples of this type of cheese.

Mold-ripened cheeses which are so pleasing to many palates develop their characteristic flavors from the activity of molds. In cheeses such as Roquefort of France, Gorgonzola of Italy, Blue Vein of Denmark and Stilton of England, as well as other lesser known varieties, the mold is Penicillium roqueforti.

In traditional production of these cheeses the mold arose from fortuitous contamination, but in commercial manufacture today it is intentionally introduced into the curd. During maturation the mold grows in the air space around the curd particles; Roquefort, Gorgonzola and Blue Vein are additionally spiked with stainless steel wires at an early stage in maturation to allow air into the curd. The mold then grows in the channels made by the wires. The open texture of the curd in Stilton provides conditions for abundant mold growth so that it is not usually spiked.

The enzymes from the growing mold diffuse into the curd and hydrolyse the milk fats to free fatty acids giving the flavors characteristic of these varieties. The fatty acids, caprylic and caproic acids, are important flavor constituents of this type of cheese. In addition, the oxidases of the molds oxidize some of the free fatty acids to ketones which are also important flavor constituents.

In the manufacture of cheeses such as Camembert and Brie, the mold is inoculated into the milk or applied to the surface of the formed curd after salting. During the maturation process the mold grows and its enzymes diffuse into the center of the curd, breaking down the milk protein as they do so. Such cheeses are matured for only about 6 weeks because if matured for longer periods some of the amino acids are reduced to ammonia which is clearly recognized when the cheese is consumed.

The white mold of Camembert cheese is Penicillium camembertii, Penicillium candidum or Penicillium caseicolum. Any green molds on the surface of a Camembert are contaminants and make the cheese unpalatable. Thus this cheese must be matured in carefully controlled rooms.

Undesirable activities of microorganisms

While mold strains such as those described above can be used to advantage, there are many that cause food spoilage and some that are a health hazard. Certain Aspergillus strains, in particular, produce mycotoxins

and some of these have been found to be carcinogenic in animals.

The lactic acid bacteria which are used to advantage in the production of fermented milks and cheese may cause defects in other products. For example, the growth of naturally occurring lactic acid bacteria in milk before processing results in defects attributable to the denaturation of casein and the production of lactic acid.

Pasteurization of milk destroys most lactic acid bacteria and pathogens, but there are some heat-resistant lactic streptococci and spore-forming organisms which survive this process. When pasteurized milk is stored for long periods it usually does not become sour as does raw milk, but goes putrid because of the proteolytic action of the heat-resistant organisms which, in raw milk, are suppressed by competition from the lactic acid bacteria that occur naturally.

In addition to organisms that can have both advantageous and disadvantageous properties, there are others which are solely spoilage organisms or are undesirable in other ways. After milk has been drawn from the cow, it usually becomes contaminated very quickly with many species of bacteria. Among these are a group of organisms known as psychrotrophs which grow at temperatures below 5° C and which have lipolytic and proteolytic activities in the milk. If these organisms are allowed to multiply in the milk before processing, the products of lipolysis and proteolysis are carried into the manufactured product, causing off-flavors.

This problem has been accentuated in recent years by the introduction of refrigerated vats for storing milk on the farm and the use of refrigerated tankers for transporting the milk. Under these conditions the mesophilic organisms such as lactic streptococci cannot compete with the psychrotrophs and the increased time of holding allows them further opportunity for growth.

The anaerobic bacteria belonging to the genus Clostridium may be normally present in milk and cause no problem, except when the milk is used in the manufacture of cheese such as Gouda, in which they may cause gas production. The deep interior of the cheese is anaerobic and since the acid and salt levels in this region are initially low, conditions are favorable for the growth of these organisms. Clostridia may also be a problem in processed cheese.

While pasteurization destroys pathogens in the milk, there are opportunities during manufacture for these and spoilage organisms to enter dairy products and to grow during processing. It is the duty of the food processor and the food handler to take every precaution with hygienic handling of food, cleaning of equipment and personal hygiene. If coagulase-positive staphylococci have an opportunity to grow in food including dairy products, they may also have the opportunity to produce enterotoxins which are not destroyed by any further processing or cooking. As many people carry these organisms in their noses and on their hands, and because other organisms such as viruses and enteric pathogens are also carried by humans, strict hygiene is essential at all times to avoid transmission from food to the consumer.

Escherichia coli is an organism which is used as an index of direct faecal pollution of water, but it cannot be regarded in the same light for food because in the processing of milk and other foods it is possible that the E. coli remains on the equipment and is simply transferred from batch to batch of food. E. coli can, together with the coliform organisms, as a group, be an index of the cleanliness of the equipment and it is in this context that it is used in the food industry.

Control of microorganisms

There are many microorganisms whose natural habitat is milk and others which are always present in air. It is by utilizing knowledge of the growth requirements of microorganisms that the processor can prevent spoilage in food. Thus processing

conditions can be controlled so that the products have a low water activity, a pH range unfavorable for microbial growth, or some other growth-limiting characteristics. For example, in butter, although the total water content is sufficient to support bacterial growth, a limit to growth is imposed by dispersing the water throughout the fat in droplets that are too small to accommodate a bacterial cell, let alone allow it to multiply. However, if the butter is stored at a temperature which allows migration and aggregation of the water droplets conditions favoring bacterial growth can arise. Addition of the salt to butter, while causing some enlargement of the water droplets, has a compensating inhibitory action on some bacteria.

Ripening of cream with aroma cultures lowers the pH of the cream and thus has a protective action against the growth of some spoilage organisms.

In cheese, bacteria multiply in the gel spaces containing water and on moist interfaces between the original curd particles. The more water that is removed by curd shrinkage during the cheese-making process the better is the keeping quality of the cheese. Soft cheeses, such as cottage cheese, have a short shelf life because of their high water content and, at the other end of the scale, cheeses such as hard, grating Parmesan may keep for several years. As discussed earlier, bacterial growth is essential in the manufacture of cheese, but because of the unfavorable conditions most bacteria die relatively early in the life of the cheese. It is the residual bacterial and natural milk enzymes which play the major part in the maturation process. The growth of contaminant molds is controlled not only by the anaerobic conditions, which are quickly established in the body of the cheese, but also by careful packaging with films of low oxygen permeability.

Low water activity of a product can be achieved in several other ways. The water may be removed by spray or roller drying as with milk powder. In sweetened condensed milk the addition of sugar combined with evaporation of water increases the percentage of total solids and gives rise to a high osmotic pressure, thereby lowering the water activity. Unlike other canned foods, sweetened condensed milk is not sterilized in a retort. The air must be excluded from the can by filling it completely as any remaining head space could result in the growth of molds and osmophilic bacteria.

Recognition of the habits of microorganisms, careful attention to the methods set down for manufacturing dairy products and observance of hygienic practices in food handling, packaging and storage are the factors which keep microorganisms under control.

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SUPPLEMENTARY FEEDING PROGRAMME*

Need for fresh look

Need

The recent recommendation of the United Nations World Food Conference "to introduce in the period 1975-1976 emergency programmes for supplementary feeding of a substantial number of malnourished children..." has focused attention of international and nongovernmental agencies and countries on this form of nutrition intervention. In view of the alarming food and nutrition situation that is existing today on a global scale, there are indications that fairly massive feeding programmes are already being thought of and undoubtedly this would need very impressive investment.

Since supplementary feeding programmes cannot be the permanent answer to the problem of malnutrition and since no government can afford to permanently subsidize such programmes on a large scale, the logical answer to the problem of malnutrition in the ultimate analysis will be to attain a level of socioeconomic development and food production and a pattern of income distribution which will make supplementary feeding unnecessary. However, till such time, it will be necessary to undertake short-term measures. It is in this context that large scale feeding programmes for children and other vulnerable segments of the population belonging to the economically weaker segments are highly desirable.

Supplementary feeding programme is possibly the oldest and most commonly practiced nutrition intervention measure. Supplying foods to children, who presumably are hungry, has an instant appeal and therefore attracts funds and favorable response from political decision makers. The relation between feeding and nutrition improvement needs no justification. Organizing such programmes is also regarded as comparatively simple requiring no technical expertise.

Different objectives

It is no wonder that programmes continue to be undertaken in various sectors, e.g., education, social-welfare and health, during

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*Nutrition Unit, World Health Organization, Geneva. Based on a discussion paper submitted to Interorganization Meetings on Expanded Supplementary Feeding Programmes for Vulnerable Groups, March 1975.

normal times. During emergencies, such programmes are undertaken mostly as relief measures. The objectives with which such programmes are planned are significantly different and vary widely according to the implementing sector. When such programmes are initiated in the education sector, the main objectives are the teaching of food habits, better enrolment, less drop-outs and absenteeism and better school performance, although the improvement in health and nutrition is an objective by implication only. During emergencies, the objective is to provide relief to the starving population whereas in the social welfare sector, such programmes have the main objective of reducing social deprivation. It is only the health sector which considers improvement in health and nutritional status as the programme's main objective. Irrespective of the objective, the commonly employed parameters for evaluation (if it is done at all) of the success of supplementary feeding programmes are those related to growth of the body - usually height and weight. The results of such evaluation are frequently disappointing. The failure to get any significant improvement in height and weight does not necessarily imply the futility of such programmes. Often, such feeding programmes, though described as "supplementary" become "substitute" feeding programmes and as such cannot be expected to produce a marked nutritional improvement.

The design of evaluation should be based on the main objective of the programme. Each feeding programme, at its initial stage, should clearly identify its principal objective, on the basis of which the terminal evaluation is to be done. If the feeding is done with social, economic or educational objectives, it is not essential to expect a significant increase in height and weight of the beneficiaries.

Health sector involvement

Health sectors have usually pioneered the implementation of supplementary feeding programmes. Many programmes in the past have received the assistance of WHO and the UNICEF. The programme basically consisted of supplying skimmed milk powder - usually 40 g per day, fed in the reconstituted form - through the health infrastructures to infants, young children, pregnant and lactating women. To ensure that the supplement reaches the target group, the health authorities insisted on spot feeding for which the beneficiaries had to come to the distribution centers daily (usually walking several miles) for a glass of milk. These daily visits made the programme unpopular within a short time. The other alternative of supplying milk powder for a week or fortnight soon led to its leakage into the market, where the product is in great demand, not only as food but for other uses as well. Even if the milk powder was consumed in the family, it was shared by other members of the family with no great nutritional impact on the target group. Due to various reasons, mostly poor logistics, the coverage of the skimmed milk feeding programme in many developing countries was largely unsatisfactory. In fact, supplementary feeding programmes in the health sector became a means to an end (e.g. to support nutrition education for women, as an adjunct for nutrition rehabilitation) and not an end in itself.

In later years, the value of 40 g of skimmed milk powder as a food supplement for the prevention of infant and child malnutrition was seriously questioned - since most of these beneficiaries were indeed having an associated, severe calorie deficiency. With the gradual withdrawal of UNICEF from the skimmed milk feeding programme, this activity of the health sector in most developing countries became insignificant. The replacement of skimmed milk powder in later years by other processed foods like CSM, did not improve the position.

There were commonly two nutritional drawbacks in such programmes. In several instances foods with high protein content were the main ingredient of the feeding programme on the presumption that protein deficiency was the only problem. The effects of such feeding were marginal. In certain

other programmes, the food donated as external aid, supplied only the processed protein rich food on the understanding that the recipient countries would be able to make up the food calories in the form of cereals and fats. In most cases, this did not materialize due to the country's financial limitations. In both types of feeding programmes the food given showed a comparative imbalance between calorie and protein, leading to insignificant nutritional benefits.

The reluctance of the health sector to be involved in the tedious day-to-day feeding programmes and record keeping was to a large extent responsible for the gradual disappearance of such programmes in the health sector. Nevertheless, the distribution system and the organization pattern of such feeding programmes through the health sector are crucial for its success.

There are some countries who have undertaken massive programmes for the supplementary feeding of pre-school children with the basic objective of nutritional protection and promotion. Special Nutrition Programmes in India under the auspices of the Social Welfare Ministry and the Milk Allowance Programme in Chile are examples. The cost for significant coverage deters many governments from undertaking such programmes, if external assistance through bilateral or international aid is not available. Integration of pre-school feeding programmes with other aspects of child care is an approach which needs urgent consideration. UNICEF's role in building up such programmes in a few countries deserves special mention.

School feeding programme

The education sector in many countries has considerable experience in programmes mainly directed to school children in the form of school meal programmes. Improvement of health and nutrition of schoolchildren is certainly not the primary objective of such programmes. World Food Programme's assistance in recent years to many countries for school meal programmes has, however, played some part in focusing attention on the need for nutritional improvement of the beneficiaries. In general, school meal programme is a "substitute" feeding programme and the supplementation is only of marginal benefit, excepting in areas where the school children come from extremely poor families. There are very few studies on the impact of school meal programmes on the nutritional status of the beneficiaries. There are still fewer to indicate that such programmes have made any significant change in the growth rate of the beneficiaries.

Notwithstanding these limitations, school feeding programmes have definite advantages in having a ready-made operational base, readily available beneficiaries and good personnel - the teachers. This institutional base should be utilized for implementing other programmes such as nutrition education and nutrition surveillance.

Emergency feeding

The supplementary feeding programmes organized during emergencies are basically relief measures and cannot be strictly regarded as nutrition intervention. In such circumstances, processed foods have an important role. Emergency situations are also invariably associated with serious health problems. Hence emergency feeding programmes should always be linked up with health protection measures.

A common disturbing feature in emergency feeding programmes is the scant attention given to the needs of children - in terms of special foods and appropriate feeding systems. At the present time a large majority of the developing countries is in a state of food and nutrition emergency. This might remain so for some time to come. It might facilitate future consideration if emergency situations can be further subdivided into "acute" disasters needing assistance for a short duration and the "chronic" emergencies, which UNICEF has rightly described as "creeping" malnutrition.

Some reservations

There is a growing feeling in certain quarters that nutrition intervention in the form of feeding programmes has not been able to achieve the objectives and in most cases the programmes are not realistic and feasible. In fact, there are substantial reservations about such measures. Among these, the following deserve serious consideration:

a) A feeding programme is by far the most expensive nutrition intervention measure. Can the cost-effectiveness be justified compared to other intervention measures? If these are short-term relief operations for mitigating severe malnutrition, as is the general impression, hard-headed economists may well be justified in questioning the wisdom of diverting large resources to such programmes.

b) With all the operational constraints in most developing countries, can supplementary feeding programmes produce any significant nutritional impact? The point may legitimately be raised as to whether or not more lasting and substantial improvement of the health and nutritional status of the people can be brought about through utilizing meager resources for the improvement of environmental sanitation, protected water supply, improved local production and conservation of foods, and other measures.

c) Coverage is always limited. Usually the programmes reach those areas where they are not so desperately needed, whereas those living in the remote outlying areas and in desperate need for such supplements do not get it for logistic reasons. The unsatisfactory coverage in pre-school feeding, compared to school feeding, is almost entirely due to logistic difficulties.

d) The operating expenditure for such programmes, e.g., storage, transport, personnel, etc. is considerable. On simple grounds of economics, this intervention may be unjustifiable.

e) While an on-the-spot feeding system ensures the intake of food by the beneficiaries, it has a great drawback in being unpopular and sometimes unrealistic in many circumstances. The school feeding is of course an exception. The other alternative is the take-home delivery system - where the amount of food required will be much larger and with the added following questions: i) Does the food reach the target child in required amount? ii) Is a portion sold to get cash money for other needs? iii) How much of the food goes for other members of the families in less need and even to pets? There are several studies in recent years to indicate the operational difficulties of both approaches.

f) A very large number of processed foods have been tried out. While these are fairly acceptable during acute emergencies, for normal supplementary feeding they are not very popular. In mounting massive feeding programmes, the choice of food is a crucial question and a satisfactory solution is often very difficult.

In the absence of clear categorical answers to these problems, one would not be in a position to justify the implementation of feeding programmes as nutrition intervention. On the other hand, the need for the nutritional protection of millions of children and other vulnerable populations is so palpable and so urgent that some form of nutrition intervention is urgently needed.

Proposals for future strategy

The approach of the World Health Organization has been all along categorical in regarding supplementary feeding programmes as just one aspect of the total approach for nutritional improvement and that feeding programmes have necessarily to be supported by other health measures which have to be adopted simultaneously. Supplementary feeding of children with high incidence of infections and infestations cannot produce the expected nutritional impact.

Feeding programmes have an instant appeal in all countries and in most situations.

Feeding by itself gives sometimes an impression of charity and hence becomes unpopular among those who are not desperately poor. Similarly, feeding without other health measures will not only be ineffective but will mean misuse of funds and efforts. The strategy should be to utilize the general appeal of a feeding programme and build around it other more fundamental needed measures to make it more appealing, comprehensive and with far more impact.

The following points need consideration during planning and implementation of supplementary feeding programmes:

1) A feeding programme is an expensive measure. Even if the food comes as an aid, the overhead costs, to be borne by the government, act as a deterrent. As such, feeding programmes, on principle, should be the basis of other associated health and mother-child care programmes. Attendance of mothers at MCH clinics always shows an upward trend whenever some food supplements are given. This would enable sharing the capital costs (e.g. operational base and personnel) for the packaged programmes.

2) For increasing coverage with the limited food, only those children or women who are at-risk should be the beneficiaries. Screening can be done on the basis of simple indicators. Feeding programmes can be utilized to form the base for simple nutritional surveillance systems.

3) Although it is common to include pregnant and lactating women among the vulnerable groups, in actual practice very little attention is given to them while all considerations are concentrated on pre-school children. Food supplements provided to the mother, instead of the infant, may in the long run be more effective by inducing better lactation and thereby preventing the risk of early weaning. All supplementary feeding programmes should therefore give due consideration to this aspect.

4) Immunization and health and nutrition education should be an obligatory part of feeding programmes. Controlling infections is the best means of ensuring the biological utilization of foods. A feeding center can be an ideal place for implementing immunization programmes.

5) Feeding programmes always attract mothers with young children, who are ideal targets for advice and service for birth spacing. Feeding programmes should be linked up with family planning programmes. This will have mutually reinforcing effects. In fact, a few pilot trials have shown that a center for child feeding utilized also for family planning services is more acceptable to the women.

6) Personnel for feeding programmes should, as far as possible, be people from the community, who can be given a very short training in the form of orientation in health, including nutrition, maternal child health, health education and family planning. This would generate a core of "primary health workers" as envisaged by both WHO and UNICEF.

This is already being adopted in several countries with supplementary feeding forming the central core of a comprehensive program.

7) While school feeding programmes can be relatively easy for implementation, infants and pre-school age children need these programmes much more than school age children. From a health and nutrition consideration, resources presently utilized for school meal programmes should gradually - in phased form - be directed for pre-school feeding.

If supplementary feeding programmes are to be mounted on a massive scale as a nutrition intervention measure, they should be given a hard critical look. There is the need to rationalize the programmes to make them more effective, less expensive and with a much broader objective.

BOOKS

Nutrition Education Research Project - 3 volumes

1. Nutrition Education Research Project - A review of literature (1970).
2. A Field Guide for Evaluation of Nutrition Education (1975).
3. Application of Field Guide for Evaluation of Nutrition Education in 3 Programs in Brazil (1976).

Office of Nutrition, Technical Assistance Bureau, Agency for International Development, Washington, D.C., USA.

The publications are the outcome of a project concerned with the evaluation of the effectiveness of nutrition education in improving food habits and nutrition in overseas programs. The lack of a simple, effective evaluation methodology for nutrition education projects in developing countries was determined to be a key constraint to the design of better, more cost-effective programs.

The first publication, in this series Nutrition Education Research Project, reviews existing literature on nutrition education from 1900 to 1970. The summary of findings and recommendations reveal, among other things, that nutrition education research has been directed more toward the purpose of disseminating nutrition information than toward the purpose of improving dietary habits. It concluded that nutrition education can be effective in improving dietary habits within carefully defined limitations and noted than an accepted and tried methodology to evaluate such programs did not exist.

The second publication, A Field Guide for Evaluation of Nutrition Education, derives from the literature review and recomendation for developing an evaluation methodology. The Field Guide is intended as an evaluation tool for planners and nutrition educators who are concerned with the cost-effectiveness of specific nutrition education programs in improving food behavior or in comparing the cost-effectiveness of different programs. The methodology was reviewed by a technical panel, modified, then field tested in Brazil, and was then modified again. The field guide is presently being further tested in Pakistan and the Philippines and the results are yet to come in.

The third publication, Application of the Field Guide for Evaluation of Nutrition Education in Three Programs in Brazil, provides the results of applying the methodology to three different educational approaches -- person-to-person, group teaching, and mass media.

ERRATA

PAG Bulletin, Vol. VI, No. 1, March 1976:

Page 47, column 1, paragraph 2 should read:

"Through IGLIC, IITA will publish annotated bibliographies and will provide a variety of specialized information services to agencies, projects, scientists and experts working on grain legumes in all parts of the world. It is intended to compile a guide or register of agencies, projects and research workers in the field of tropical grain legumes. A new quarterly publication..."

PAG Bulletin, Vol. VI, No. 2, June 1976:

Page 38, column 2, line 18, please add:

The Director of the project would like the following names of co-authors to be mentioned: Avinash C. Kansra, N. Srinivasan, Indira Varadarajan, Arvind G. Shingwekar, Ranjna Seth, R.S. Mathur, and V. Bhargava.

PAG DOCUMENTS*

Statements

No.	Title	Year
2	Recommendation on aflatoxin (S)	1969
3	Nature and magnitude of the protein problem (S)	1971
4	Single cell protein (F, S)	1970
5	Marketing and distribution of protein-rich foods (S)	1971
6	Milk substitutes (S)	1970
7	Recommendation on prevention of food losses and protein-calorie malnutrition (S)	1969
8	Plant improvement by genetic means	1970
9	Amino acid fortification of foods (F, S)	1970
10	A systems approach to the formulation and evaluation of nutrition intervention programs (S)	1970
11	Leaf protein concentrate (S)	1970
12	The world protein problem: research and development needs (S)	1972
13a	Review of the specific proposals contained in ACAST report "International Action to Avert the Impending Protein Crisis", United Nations, 1968 (S)	1971
14	Marketing of conventional foods (S)	1971
15	Popular participation and community involvement in nutrition improvement programs (S)	1971
16	The potential of fish protein concentrate for developing countries (F, S)	1971
17	Low lactase activity and milk intake (F, S)	1972
18	Relationship of pre- and postnatal malnutrition in children to mental development, learning and behavior (S)	1972
19	Maintenance and improvement of nutritional quality of protein foods (S)	1972
20	The "protein problem" (F, S)	1973
21	Specifications for solvents (S)	1972
22	Upgrading human nutrition through the improvement of food legumes (F, S)	1973
23	Promotion of special foods (infant formula and processed protein foods) for vulnerable groups (F, S)	1973
24	The Green Revolution and protein supplies	1973
25	The global maldistribution of protein: a growing trend (F)	1973
26	Food and nutrition considerations in national economic planning	1973
27	Mass communications in nutrition education (F, S)	1974
28	Issues for the World Food Conference, the PAG view (F, S)	1975

Guidelines

2	Preparation of food-quality groundnut flour	1970
4	Preparation of edible cottonseed protein concentrate	1970
5	Edible, heat-processed soy grits and flour (F, S)	1969
6	Preclinical testing of novel sources of protein (F)	1972
7	Human testing of supplementary food mixtures (F)	1972
8	Protein-rich mixtures for use as weaning foods (F)	1972
9	Fish protein concentrate	1971
10	Marketing of protein-rich foods in developing countries (F, S)	1971
11	Sanitary production and use of dry protein foods (F, S)	1972
12	Production of single cell protein for human consumption (F)	1972
13	Preparation of milk substitutes of vegetable origin and toned milk containing vegetable protein	1972
14	Preparation of defatted edible sesame flour	1972
15	Nutritional and safety aspects of novel protein sources for animal feeding (F, S)	1974
16	Protein methods for cereal breeders as related to human nutritional requirements (F, S)	1975

*The letters in parentheses indicate those documents that have been translated into French (F) or Spanish (S) or both (F, S).

Most PAG Statements and Guidelines have been published in the PAG Bulletin. They may be used, quoted or reprinted freely.

PAG DOCUMENTS

1. Documents on single cell protein issued by PAG. Contents: 1976
   a) Statement No. 4 on SCP, 1972
   b) Report of 2nd PAG ad hoc working group meeting, 1971
   c) Report of 3rd PAG ad hoc working group meeting, 1973
   d) Report of 4th PAG ad hoc working group meeting, 1973
   e) Report of 5th PAG ad hoc working group meeting on clinical evaluation and acceptable nucleic acid levels of SCP for human consumption, 1975
   f) Guideline No. 6 for Preclinical testing of novel sources of protein, 1972
   g) Guideline No. 7 for Human testing of supplementary food mixtures, 1972
   h) Guideline No. 12 on the Production of SCP for human consumption, 1972
   i) Guideline No. 15 on Nutrition and safety aspects of novel protein sources for animal feeding, 1974
2. Documents on feeding the preschool child issued by PAG ad hoc working group meetings (1969-1975). Contents: 1976
   a) Report of 2nd PAG ad hoc working group meeting, 1970
   b) Report of 3rd PAG ad hoc working group meeting, 1971
   c) Report of PAG ad hoc working group meeting on milk intolerance - nutritional implications, 1971
   d) Report of 4th PAG ad hoc working group meeting, 1972
   e) Report of 5th PAG ad hoc working group meeting, 1975
   f) PAG Statement No. 17 - Low lactase activity and milk intake, 1972
   g) PAG Statement No. 18 - Relationship of pre- and postnatal malnutrition in children to mental development, learning and behavior, 1972
   h) PAG Guideline No. 8 - Protein-rich mixtures for use as weaning foods, 1972
3. Mass communication: Report of PAG ad hoc working group meeting, New York 1975
4. Overcoming problems in infant and young child feeding practices: PAG regional seminar, Singapore 1974
5. Pediatrician/infant food industry seminar, New York 1973
6. Nutritional improvement of food legumes by breeding 1973
7. Manual on feeding infants and young children by Cameron and Hofvander 1972
   (2nd edition: 1976)

PROTEIN-CALORIE ADVISORY GROUP

The Protein-Calorie Advisory Group of the United Nations System (PAG) is an interdisciplinary committee of internationally-recognized experts who advise the United Nations and its agencies on technical, economic, educational, social and other related aspects of global malnutrition problems and the broad programs and new areas of activity needed for combating them. Since its inception in 1955, the PAG has emphasized protein-calorie malnutrition as a primary and continuing threat to the health and survival of infants and young children in the developing countries and has played an active role in promoting the development of novel and locally-available protein resources for the developing world. The PAG also reacts to socioeconomic considerations, trends in world food supply and consumption and the need for governmental initiatives and priorities in dealing with these problems.

The PAG is sponsored by the Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Bank for Reconstruction and Development (IBRD), and the United Nations.

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